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SIX-MONTH IMPLEMENTATION GUIDELINE

The *United States Pharmacopeia–National Formulary* and its supplements become official six months after being released to the public. The *USP–NF*, which is released on November 1 of each year, becomes official on May 1 of the following year. This six-month implementation timing gives users more time to bring their methods and procedures into compliance with new and revised *USP–NF* requirements.

The table below describes the official dates of the *USP–NF* and its supplements. The 2016 *USP 39–NF 34*, and its supplements, *Interim Revision Announcements (IRAs)* and *Revision Bulletins* to that edition, will be official until May 1, 2017, at which time the *USP 40–NF 35* becomes official.

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GENERAL NOTICES AND REQUIREMENTS

The *General Notices and Requirements* section (the *General Notices*) presents the basic assumptions, definitions, and default conditions for the interpretation and application of the *United States Pharmacopeia* (USP) and the *National Formulary* (NF).

Requirements stated in these *General Notices* apply to all articles recognized in the USP and NF (the "compendia") and to all general chapters unless specifically stated otherwise.

1. TITLE AND REVISION

The full title of this publication (consisting of four volumes and including its *Supplements*), is *The Pharmacopeia of the United States of America*, Fortieth Revision and the *National Formulary*, Thirty-Fifth Edition. These titles may be abbreviated to USP 40, to NF 35, and to USP 40–NF 35. The *United States Pharmacopeia*, Fortieth Revision, and the *National Formulary*, Thirty-Fifth Edition, supersede all earlier revisions. Where the terms "USP," "NF," or "USP–NF" are used without further qualification during the period in which these compendia are official, they refer only to USP 40, NF 35, and any *Supplement(s)* thereto. The same titles, with no further distinction, apply equally to print or electronic presentation of these contents. Although USP and NF are published under one cover and share these *General Notices*, they are separate compendia.

This revision is official beginning May 1, 2017 unless otherwise indicated in specific text.

Supplements to USP and NF are published periodically.

Accelerated Revisions, published periodically on the *Official Text* section of USP's website (<http://www.usp.org/usp-nf/official-text>), are designed to make revisions official more quickly than through the routine process for publishing standards in the USP–NF. *Interim Revision Announcements* are Accelerated Revisions to USP and NF that contain official revisions and their effective dates.

Revision Bulletins are Accelerated Revisions to official text or postponements that require expedited publication. They generally are official immediately unless otherwise specified in the *Revision Bulletin*.

Errata are Accelerated Revisions representing corrections to items erroneously published. Announcements of the availability of new USP Reference Standards and announcements of tests or procedures that are held in abeyance pending availability of required USP Reference Standards are also available on the "Official Text" tab of USP's website.

2. OFFICIAL STATUS AND LEGAL RECOGNITION

2.10. Official Text

Official text of the USP and NF is published in the USP–NF Online (www.uspnf.com) in the edition identified as "CURRENTLY OFFICIAL" and in Accelerated Revisions that supersede the USP–NF Online as described below.

Routine revisions are published in the USP–NF Online and become official on the date indicated, usually six months after publication. Accelerated Revisions supersede the USP–NF Online and become official on the date indicated. Links to Accelerated Revisions on the USP website can be found in any superseded monograph or general chapter in the USP–NF Online.

Print and USB flash drive versions of the USP and NF also are available. Routine revisions are provided with the same timing as the USP–NF Online. Official text published in *Supplements* supersedes that in the previously published print or

USB flash drive versions of USP–NF. These versions also are superseded by Accelerated Revisions as described above.

In the event of any disparity between the print or USB flash drive versions and the USP–NF Online, the USP–NF Online will be deemed to apply.

2.20. Official Articles

An *official article* is an article that is recognized in USP or NF. An article is deemed to be recognized and included in a compendium when a monograph for the article is published in the compendium and an official date is generally or specifically assigned to the monograph.

The title specified in a monograph is the *official title* for such article. Other names considered to be synonyms of the official titles may not be used as substitutes for official titles.

Official articles include both *official substances* and *official products*. An *official substance* is a drug substance, excipient, dietary ingredient, other ingredient, or component of a finished device for which the monograph title includes no indication of the nature of the finished form.

An *official product* is a drug product, dietary supplement, compounded preparation, or finished device for which a monograph is provided.

2.30. Legal Recognition

The USP and NF are recognized in the laws and regulations of many countries throughout the world. Regulatory authorities may enforce the standards presented in the USP and NF, but because recognition of the USP and NF may vary by country, users should understand applicable laws and regulations. In the United States under the Federal Food, Drug, and Cosmetic Act (FDCA), both USP and NF are recognized as official compendia. A drug with a name recognized in USP–NF must comply with compendial identity standards or be deemed adulterated, misbranded, or both. See, e.g., FDCA § 501(b) and 502(e)(3)(b); also FDA regulations, 21 CFR § 299.5(a&b). To avoid being deemed adulterated, such drugs must also comply with compendial standards for strength, quality, and purity, unless labeled to show all respects in which the drug differs. See, e.g., FDCA § 501(b) and 21 CFR § 299.5(c). In addition, to avoid being deemed misbranded, drugs recognized in USP–NF must also be packaged and labeled in compliance with compendial standards. See FDCA § 502(g).

A dietary supplement represented as conforming to specifications in USP will be deemed a misbranded food if it fails to so conform. See FDCA § 403(s)(2)(D).

Enforcement of USP standards is the responsibility of FDA and other government authorities in the U.S. and elsewhere. USP has no role in enforcement.

Change to read:

3. CONFORMANCE TO STANDARDS

3.10. Applicability of Standards

Standards for an article recognized in the compendia (USP–NF) are expressed in the article's monograph, applicable general chapters, and *General Notices*. The identity, strength, quality, and purity of an article are determined by the official tests, procedures, and acceptance criteria, and other requirements incorporated in the monograph, in applicable general chapters, or in the *General Notices*. "Applicable general chapters" means general chapters numbered

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below 1000 or above 2000 that are made applicable to an article through reference in *General Notices*, a monograph, or another applicable general chapter numbered below 1000. Where the requirements of a monograph differ from the requirements specified in these *General Notices* or an applicable general chapter, the monograph requirements apply and supersede the requirements of the *General Notices* or applicable general chapters, whether or not the monograph explicitly states the difference.

General chapters numbered 1000 to 1999 are for informational purposes only. They contain no mandatory tests, assays, or other requirements applicable to any official article, regardless of citation in a general chapter numbered below 1000, a monograph, or these *General Notices*. General chapters numbered above 2000 apply only to articles that are intended for use as dietary ingredients and dietary supplements. General chapter citations in *NF* monographs refer to *USP* general chapters.

Early adoption of revised standards in advance of the official date is allowed by *USP* unless specified otherwise at the time of publication. Where revised standards for an existing article have been published as final approved "official text" (as approved in section 2.10 *Official Text*) but have not yet reached the official date (six months after publication, unless otherwise specified; see "official date", section 2.20 *Official Articles*), compliance with the revised standard shall not preclude a finding or indication of conformance with compendial standards, unless *USP* specifies otherwise by prohibiting early adoption in a particular standard.

The standards in the relevant monograph, general chapter(s), and *General Notices* apply at all times in the life of the article from production to expiration. It is also noted that the manufacturer's specifications, and manufacturing practices (e.g., Quality by Design, Process Analytical Technology, and Real Time Release Testing initiatives), generally are followed to ensure that the article will comply with compendial standards until its expiration date, when stored as directed. Every compendial article in commerce shall be so constituted that when examined in accordance with these assays and test procedures, it meets all applicable pharmacopeial requirements (*General Notices*, monographs, and general chapters). Thus, any official article is expected to meet the compendial standards if tested, and any official article actually tested as directed in the relevant monograph must meet such standards to demonstrate compliance.

Some tests, such as those for Dissolution and Uniformity of Dosage Units, require multiple dosage units in conjunction with a decision scheme. These tests, albeit using a number of dosage units, are in fact one determination. These procedures should not be confused with statistical sampling plans. The similarity to statistical procedures may seem to suggest an intent to make inference to some larger group of units, but in all cases, statements about whether the compendial standard is met apply only to the units tested. Repeats, replicates, statistical rejection of outliers, or extrapolations of results to larger populations, as well as the necessity and appropriate frequency of batch testing, are neither specified nor proscribed by the compendia; such decisions are based on the objectives of the testing. Frequency of testing and sampling are left to the preferences or direction of those performing compliance testing, and other users of *USP-NF*, including manufacturers, buyers, or regulatory authorities.

Official products are prepared according to recognized principles of good manufacturing practice and from ingredi-

ents that meet *USP* or *NF* standards, where standards for such ingredients exist (for dietary supplements, see section 3.10.20).

Official substances are prepared according to recognized principles of good manufacturing practice and from ingredients complying with specifications designed to ensure that the resultant substances meet the requirements of the compendial monographs.

3.10.10. Applicability of Standards to Drug Products, Drug Substances, and Excipients

The applicable *USP* or *NF* standard applies to any article marketed in the United States that (1) is recognized in the compendium and (2) is intended or labeled for use as a drug or as an ingredient in a drug. Such articles (drug products, drug substances, and excipients) include both human drugs (whether dispensed by prescription, "over the counter," or otherwise), as well as animal drugs. The applicable standard applies to such articles whether or not the added designation "*USP*" or "*NF*" is used. The standards apply equally to articles bearing the official titles or names derived by transposition of the definitive words of official titles or transposition in the order of the names of two or more active ingredients in official titles, or where there is use of synonyms with the intent or effect of suggesting a significant degree of identity with the official title or name.

3.10.20. Applicability of Standards to Medical Devices, Dietary Supplements, and Their Components and Ingredients

An article recognized in *USP* or *NF* shall comply with the compendial standards if the article is a medical device, component intended for a medical device, dietary supplement, dietary ingredient, or other ingredient that is intended for incorporation into a dietary supplement, and is labeled as conforming to the *USP* or *NF*.

Generally, dietary supplements are prepared from ingredients that meet *USP*, *NF*, or *Food Chemicals Codex* standards. Where such standards do not exist, substances may be used in dietary supplements if they have been shown to be of acceptable food grade quality using other suitable procedures.

*3.10.30. Applicability of Standards to the Practice of Compounding (New)

USP compounding practice standards, *Pharmaceutical Compounding—Nonsterile Preparations* (795) and *Pharmaceutical Compounding—Sterile Preparations* (797), as appropriate, apply to compounding practice or activity regardless of whether a monograph exists for the compounded preparation or these chapters are referenced in such a monograph. In the United States, (795) and (797) are not applicable to drugs compounded by entities registered with FDA as outsourcing facilities as defined by FDCA § 503B, because such facilities are required to comply with FDA's current good manufacturing practice requirements. Compounded preparations, including drug products compounded by outsourcing facilities, may also be subject to applicable monographs; see section 2.20 *Official Articles* and section 4.10 *Monographs*. ▲*USP40*

3.20. Indicating Conformance

A drug product, drug substance, or excipient may use the designation "*USP*" or "*NF*" in conjunction with its official title or elsewhere on the label only when (1) a monograph

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is provided in the specified compendium and (2) the article complies with the identity prescribed in the specified compendium.

When a drug product, drug substance, compounded preparation, or excipient differs from the relevant *USP* or *NF* standard of strength, quality, or purity, as determined by the application of the tests, procedures, and acceptance criteria set forth in the relevant compendium, its difference shall be plainly stated on its label.

When a drug product, drug substance, compounded preparation, or excipient fails to comply with the identity prescribed in *USP* or *NF* or contains an added substance that interferes with the prescribed tests and procedures, the article shall be designated by a name that is clearly distinguishing and differentiating from any name recognized in *USP* or *NF*.

A medical device, dietary supplement, or ingredient or component of a medical device or dietary supplement may use the designation "USP" or "NF" in conjunction with its official title or elsewhere on the label only when (1) a monograph is provided in the specified compendium and (2) the article complies with the monograph standards and other applicable standards in that *USP* or *NF* compendium.

The designation "USP" or "NF" on the label may not and does not constitute an endorsement by USP and does not represent assurance by USP that the article is known to comply with the relevant standards. USP may seek legal redress if an article purports to be or is represented as an official article in one of USP's compendia and such claim is determined by USP not to be made in good faith.

The designation "USP-NF" may be used on the label of an article provided that the label also bears a statement such as "Meets *NF* standards as published by USP," indicating the particular compendium to which the article purports to apply.

When the letters "USP," "NF," or "USP-NF" are used on the label of an article to indicate compliance with compendial standards, the letters shall appear in conjunction with the official title of the article. The letters are not to be enclosed in any symbol such as a circle, square, etc., and shall appear in capital letters.

If a dietary supplement does not comply with all applicable compendial requirements but contains one or more dietary ingredients or other ingredients that are recognized in *USP* or *NF*, the individual ingredient(s) may be designated as complying with *USP* or *NF* standards or being of *USP* or *NF* quality provided that the designation is limited to the individual ingredient(s) and does not suggest that the dietary supplement complies with *USP* standards.

4. MONOGRAPHS AND GENERAL CHAPTERS

4.10. Monographs

Monographs set forth the article's name, definition, specification, and other requirements related to packaging, storage, and labeling. The specification consists of tests, procedures, and acceptance criteria that help ensure the identity, strength, quality, and purity of the article. For general requirements relating to specific monograph sections, see section 5 *Monograph Components*.

Because monographs may not provide standards for all relevant characteristics, some official substances may conform to the *USP* or *NF* standard but differ with regard to nonstandardized properties that are relevant to their use in specific preparations. To assure substitutability in such instances, users may wish to ascertain functional equivalence or determine such characteristics before use.

4.10.10. Applicability of Test Procedures

A single monograph may include more than one test, procedure, and/or acceptance criterion for the same attribute. Unless otherwise specified in the monograph, all tests are requirements. In some cases, monograph instructions allow the selection of tests that reflect attributes of different manufacturers' articles, such as different polymorphic forms, impurities, hydrates, and dissolution. Monograph instructions

indicate the tests, procedures, and/or acceptance criteria to be used and the required labeling.

The order in which the tests are listed in the monograph is based on the order in which they are approved by the relevant Expert Committee for inclusion in the monograph. Test 1 is not necessarily the test for the innovator or for the reference product. Depending on monograph instructions, a labeling statement is not typically required if Test 1 is used.

4.10.20. Acceptance Criteria

The acceptance criteria allow for analytical error, for unavoidable variations in manufacturing and compounding, and for deterioration to an extent considered acceptable under practical conditions. The existence of compendial acceptance criteria does not constitute a basis for a claim that an official substance that more nearly approaches 100 percent purity "exceeds" compendial quality. Similarly, the fact that an article has been prepared to tighter criteria than those specified in the monograph does not constitute a basis for a claim that the article "exceeds" the compendial requirements.

An official product shall be formulated with the intent to provide 100 percent of the quantity of each ingredient declared on the label. Where the minimum amount of a substance present in a dietary supplement is required by law to be higher than the lower acceptance criterion allowed for in the monograph, the upper acceptance criterion contained in the monograph may be increased by a corresponding amount.

The acceptance criteria specified in individual monographs and in the general chapters for compounded preparations are based on such attributes of quality as might be expected to characterize an article compounded from suitable bulk drug substances and ingredients, using the procedures provided or recognized principles of good compounding practice, as described in these compendia.

4.20. General Chapters

Each general chapter is assigned a number that appears in angle brackets adjacent to the chapter name (e.g., *Chromatography* (621)). General chapters may contain the following:

- Descriptions of tests and procedures for application through individual monographs,
- Descriptions and specifications of conditions and practices for pharmaceutical compounding,
- General information for the interpretation of the compendial requirements,
- Descriptions of general pharmaceutical storage, dispensing, and packaging practices, or
- General guidance to manufacturers of official substances or official products.

When a general chapter is referenced in a monograph, acceptance criteria may be presented after a colon.

Some chapters may serve as introductory overviews of a test or of analytical techniques. They may reference other general chapters that contain techniques, details of the procedures, and, at times, acceptance criteria.

Change to read:

5. MONOGRAPH COMPONENTS

5.10. Molecular Formula

The use of the molecular formula for the active ingredient(s) named in defining the required strength of a compendial article is intended to designate the chemical entity or entities, as given in the complete chemical name of the article, having absolute (100 percent) purity.

5.20. Added Substances

Added substances are presumed to be unsuitable for inclusion in an official article and therefore prohibited, if their presence impairs the bioavailability, therapeutic efficacy, or safety of the official article; or they interfere with the assays and tests prescribed for determining compliance with the

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compendial standards (see section 3.20 *Indicating Conformance*).

The air in a container of an official article may, where appropriate, be evacuated or be replaced by carbon dioxide, helium, argon, or nitrogen, or by a mixture of these gases. The use of such gas need not be declared in the labeling.

5.20.10. Added Substances in Official Substances

Official substances may contain only the specific added substances that are permitted by the individual monograph. Such added substances shall not exceed the quantity required for providing their intended effect. Where such addition is permitted, the label shall indicate the name(s) and amount(s) of any added substance(s).

5.20.20. Added Substances (Excipients and Ingredients) in Official Products

Suitable substances and excipients such as antimicrobial agents, pharmaceutical bases, carriers, coatings, flavors, preservatives, stabilizers, and vehicles may be added to an official product to enhance its stability, usefulness, or elegance, or to facilitate its preparation, unless otherwise specified in the individual monograph.

Added substances and excipients employed solely to impart color may be incorporated into official products other than those intended for parenteral or ophthalmic use, in accordance with the regulations pertaining to the use of colors issued by the U.S. Food and Drug Administration (FDA), provided such added substances or excipients are otherwise appropriate in all respects. (See also *Injections and Implanted Drugs Products* (1), *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Vehicles and added substances, Added substances*.) ^{▲USP40}

The proportions of the substances constituting the base in ointment and suppository products and preparations may be varied to maintain a suitable consistency under different climatic conditions, provided that the concentrations of active ingredients are not varied and provided that the bioavailability, therapeutic efficacy, and safety of the preparation are not impaired.

5.20.20.1. In Compounded Preparations

Compounded preparations for which a complete composition is given shall contain only the ingredients named in the formulas unless specifically exempted herein or in the individual monograph. Deviation from the specified processes or methods of compounding, although not from the ingredients or proportions thereof, may occur provided that the finished preparation conforms to the relevant standards and to preparations produced by following the specified process.

Where a monograph for a compounded preparation calls for an ingredient in an amount expressed on the dried basis, the ingredient need not be dried before use if due allowance is made for the water or other volatile substances present in the quantity taken.

Specially denatured alcohol formulas are available for use in accordance with federal statutes and regulations of the Internal Revenue Service. A suitable formula of specially denatured alcohol may be substituted for Alcohol in the manufacture of official preparations intended for internal or topical use, provided that the denaturant is volatile and does not remain in the finished product. A preparation that is intended for topical application to the skin may contain specially denatured alcohol, provided that the denaturant is either a usual ingredient in the preparation or a permissible added substance; in either case the denaturant shall be identified on the label of the topical preparation. Where a process is given in the individual monograph, any preparation compounded using denatured alcohol shall be identical to that prepared by the monograph process.

5.20.20.2. In Dietary Supplements

Additional ingredients may be added to dietary supplement products provided that the additional ingredients: (1) comply with applicable regulatory requirements; and (2) do

not interfere with the assays and tests prescribed for determining compliance with compendial standards.

5.30. Description and Solubility

Only where a quantitative solubility test is given in a monograph and is designated as such is it a test for purity.

A monograph may include information regarding the article's description. Information about an article's "description and solubility" also is provided in the reference table *Description and Relative Solubility of USP and NF Articles*. The reference table merely denotes the properties of articles that comply with monograph standards. The reference table is intended primarily for those who use, prepare, and dispense drugs and/or related articles. Although the information provided in monographs and the information in the reference table may indirectly assist in the preliminary evaluation of an article, it is not intended to serve as a standard or test for purity.

The approximate solubility of a compendial substance is indicated by one of the following descriptive terms:

Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1,000
Very slightly soluble	From 1,000 to 10,000
Practically insoluble, or Insoluble	Greater than or equal to 10,000

5.40. Identity

A compendial test titled *Identity* or *Identification* is provided as an aid in verifying the identity of articles as they are purported to be, e.g., those taken from labeled containers, and to establish whether it is the article named in USP-NF. The *Identity* or *Identification* test for a particular article may consist of one or more procedures. When a compendial test for *Identity* or *Identification* is undertaken, all requirements of all specified procedures in the test must be met to satisfy the requirements of the test. Failure of an article to meet all the requirements of a prescribed *Identity* or *Identification* test (i.e., failure to meet the requirements of all of the specified procedures that are components of that test) indicates that the article is mislabeled and/or adulterated.

5.50. Assay

Assay tests for compounded preparations are not intended for evaluating a compounded preparation before dispensing, but instead are intended to serve as the official test in the event of a question or dispute regarding the preparation's conformance to official standards.

5.50.10. Units of Potency (Biological)

For substances that cannot be completely characterized by chemical or physical means or that need confirmation of functionality or tertiary structure, it may be necessary to express quantities of biological activity in units of biological potency, each defined by an authoritative, designated reference standard. In cases where international reference materials have been discontinued, international units of potency may be defined in terms of molecular mass, such as in the cases of vitamins A, D, and E.

Where available, World Health Organization (WHO) international biological standards define the International Units (IU). USP monographs refer to the units assigned by USP Reference Standards either directly as International Units (IU) or as "USP Units." For some biological products, units of potency are value assigned against a corresponding U.S. Standard established by FDA, whether or not International Units or USP Units have been defined (see *Biologics* (1041)). Note that product-related labeling, e.g., on containers, need not use the full phrase "USP [product name] Units" that

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appears in many USP monograph labeling sections. The term "USP Units" can be used on product labeling consistent with USP compendial requirements, provided it is clear from the context that the volume is stated in terms of USP [product name] Units. In such circumstances it should be clear that "USP Units" and "USP [product name] Units" share the same meaning.

5.60. Impurities and Foreign Substances

Tests for the presence of impurities and foreign substances are provided to limit such substances to amounts that are unobjectionable under conditions in which the article is customarily employed (see also *Impurities in Drug Substances and Drug Products* (1086)).

Nonmonograph tests and acceptance criteria suitable for detecting and controlling impurities that may result from a change in the processing methods or that may be introduced from external sources should be employed in addition to the tests provided in the individual monograph, where the presence of the impurity is inconsistent with applicable good manufacturing practices or good pharmaceutical practices.

5.60.10. Other Impurities in USP and NF Articles

If a USP or NF monograph includes an assay or organic impurity test based on chromatography, other than a test for residual solvents, and that monograph procedure does not detect an impurity present in the substance, the amount and identity of the impurity, where both are known, shall be stated in the labeling (certificate of analysis) of the official substance, under the heading *Other Impurity(ies)*.

The presence of any unlabeled other impurity in an official substance is a variance from the standard if the content is 0.1% or greater. The sum of all *Other Impurities* combined with the monograph-detected impurities may not exceed 2.0% (see *Ordinary Impurities* (466)), unless otherwise stated in the monograph.

The following categories of drug substances are excluded from *Other Impurities* requirements:

- Fermentation products and semi-synthetics derived therefrom,
- Radiopharmaceuticals,
- Biologics,
- Biotechnology-derived products,
- Peptides,
- Herbals, and
- Crude products of animal or plant origin.

Any substance known to be toxic shall not be listed under *Other Impurities*.

5.60.20. Residual Solvents in USP and NF Articles

All USP and NF articles are subject to relevant control of residual solvents, even when no test is specified in the individual monograph. If solvents are used during production, they must be of suitable quality. In addition, the toxicity and residual level of each solvent shall be taken into consideration, and the solvents limited according to the principles defined and the requirements specified in *Residual Solvents* (467), using the general methods presented therein or other suitable methods.

5.60.30. Elemental Impurities in USP Drug Products and Dietary Supplements

Effective January 1, 2018, elemental impurities will be controlled in official drug products according to the principles defined and requirements specified in *Elemental Impurities—Limits* (232). Effective January 1, 2018, elemental contaminants are controlled in official dietary supplements according to the principles defined and requirements specified in *Elemental Contaminants in Dietary Supplements* (2232). Also effective January 1, 2018, *Heavy Metals* (231) will be omitted and all references to it in general chapters and monographs will be deleted. Early adoption of the requirements in (232) and (2232) are permitted by USP, and if (232) or (2232), as applicable, is fully implemented with respect to a particular drug product or dietary supplement in advance of the January 1, 2018 date, that product and its ingredients will no longer need to comply with applicable

(231) requirements to be considered by USP to be in conformance with USP–NF requirements.

5.70. Performance Tests

Where content uniformity determinations have been made using the same analytical methodology specified in the Assay, with appropriate allowances made for differences in sample preparation, the average of all of the individual content uniformity determinations may be used as the Assay value.

5.80. USP Reference Standards

USP Reference Standards are authentic specimens that have been approved as suitable for use as comparison standards in USP or NF tests and assays. (See *USP Reference Standards* (11).) Where USP or NF tests or assays call for the use of a USP Reference Standard, only those results obtained using the specified USP Reference Standard are conclusive. Where a procedure calls for the use of a compendial article rather than for a USP Reference Standard as a material standard of reference, a substance meeting all of the compendial monograph requirements for that article shall be used. If any new USP or NF standard requires the use of a new USP Reference Standard that is not yet available, that portion of the standard containing the requirement shall not be official until the specified USP reference material is available.

Unless a Reference Standard label bears a specific potency or content, assume the Reference Standard is 100.0% pure in the official application. Unless otherwise directed in the procedure in the individual monograph or in a general chapter, USP Reference Standards are to be used in accordance with the instructions on the label of the Reference Standard.

Change to read:

6. TESTING PRACTICES AND PROCEDURES

6.10. Safe Laboratory Practices

In performing compendial procedures, safe laboratory practices shall be followed, including precautionary measures, protective equipment, and work practices consistent with the chemicals and procedures used. Before undertaking any procedure described in the compendia, the analyst should be aware of the hazards associated with the chemicals and the techniques and means of protecting against them. These compendia are not designed to describe such hazards or protective measures.

6.20. Automated Procedures

Automated and manual procedures employing the same basic chemistry are considered equivalent.

6.30. Alternative and Harmonized Methods and Procedures

Alternative methods and/or procedures may be used if they provide advantages in terms of accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction, or in other special circumstances. Such alternative procedures and methods shall be validated as described in *Validation of Compendial Procedures* (1225) and must be shown to give equivalent or better results. Only those results obtained by the methods and procedures given in the *compendia* ^{USP40} are conclusive.

Alternative procedures should be submitted to USP for evaluation as a potential replacement or addition to the standard (see section 4.10 *Monographs*).

Certain general chapters contain a statement that the text in question is harmonized with the corresponding text of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia* and that these texts are interchangeable. Therefore, if a substance or preparation is found to comply with a requirement using an interchangeable method or procedure from one of these pharmacopoeias, it should comply with the requirements of the USP–NF. When a difference appears, or in the event of dispute, only the result obtained by the method and/or procedure given in the USP–NF is conclusive.

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6.40. Dried, Anhydrous, Ignited, or Solvent-Free Basis

All calculations in the compendia assume an "as-is" basis unless otherwise specified.

Test procedures may be performed on the undried or unignited substance and the results calculated on the dried, anhydrous, or ignited basis, provided a test for *Loss on Drying*, or *Water Determination*, or *Loss on Ignition*, respectively, is given in the monograph. Where the presence of moisture or other volatile material may interfere with the procedure, previous drying of the substance is specified in the individual monograph and is obligatory.

The term "solvent-free" signifies that the calculation shall be corrected for the presence of known solvents as determined using the methods described in (467) unless a test for limit of organic solvents is provided in the monograph.

The term "previously dried" without qualification signifies that the substance shall be dried as directed under *Loss on Drying* (731) or *Water Determination* (921) (gravimetric determination).

Where drying in vacuum over a desiccant is directed, a vacuum desiccator, a vacuum drying pistol, or other suitable vacuum drying apparatus shall be used.

6.40.10. Ignite to Constant Weight

"Ignite to constant weight" means that ignition shall be continued at $800 \pm 25^\circ$, unless otherwise indicated, until two consecutive weighings, the second of which is taken after an additional period appropriate to the nature and quantity of the residue, do not differ by more than 0.50 mg per g of substance taken.

6.40.20. Dried to Constant Weight

"Dried to constant weight" means that drying shall be continued until two consecutive weighings, the second of which is taken after an additional drying period appropriate to the nature and quantity of the residue, do not differ by more than 0.50 mg per g of substance taken.

6.50. Preparation of Solutions**6.50.10. Filtration**

Where a procedure gives direction to "filter" without further qualification, the liquid shall be passed through suitable filter paper or equivalent device until the filtrate is clear. Due to the possibility of filter effects, the initial volumes of a filtrate may be discarded.

6.50.20. Solutions

Unless otherwise specified, all solutions shall be prepared with Purified Water. Solutions for quantitative measures shall be prepared using accurately weighed or accurately measured analytes (see section 8.20 *About*).

An expression such as "(1 in 10)" means that 1 part by volume of a liquid shall be diluted with, or 1 part by weight of a solid shall be dissolved in, a sufficient quantity of the diluent or solvent to make the volume of the finished solution 10 parts by volume. An expression such as "(20:5:2)" means that the respective numbers of parts, by volume, of the designated liquids shall be mixed, unless otherwise indicated.

6.50.20.1. Adjustments to Solutions

When a specified concentration is called for in a procedure, a solution of other normality or molarity may be used, provided that allowance is made for the difference in concentration and that the change does not increase the error of measurement.

Proportionately larger or smaller quantities than the specified weights and volumes of assay or test substances and Reference Standards may be taken, provided the measurement is made with at least equivalent accuracy.

Unless otherwise indicated, analyte concentrations shall be prepared to within ten percent (10%) of the indicated value. In the case in which a procedure is adapted to the working range of an instrument, solution concentrations may differ from the indicated value by more than ten percent (10%), with appropriate changes in associated calculations. Any changes shall fall within the validated range of the instrument.

When adjustment of pH is indicated with either an acid or base and the concentration is not indicated, appropriate concentrations of that acid or base may be used.

6.50.20.2. Test Solutions

Information on Test Solutions (TS) is provided in the *Test Solutions* portion of the *Reagents, Indicators, and Solutions* section of the USP-NF. Use of an alternative Test Solution or a change in the Test Solution used may require validation.

6.50.20.3. Indicator Solutions

Where a procedure specifies the use of an indicator TS, approximately 0.2 mL, or 3 drops, of the solution shall be added unless otherwise directed.

6.60. Units Necessary to Complete a Test

Unless otherwise specified, a sufficient number of units to ensure a suitable analytical result shall be taken.

6.60.10. Tablets

Where the procedure of a Tablet monograph directs to weigh and finely powder not fewer than a given number of Tablets, a counted number of Tablets shall be weighed and reduced to a powder. The portion of the powdered Tablets taken shall be representative of the whole Tablets and shall, in turn, be weighed accurately.

6.60.20. Capsules

Where the procedure of a Capsule monograph gives direction to remove, as completely as possible, the contents of not fewer than a given number of the Capsules, a counted number of Capsules shall be carefully opened and the contents quantitatively removed, combined, mixed, and weighed accurately. The portion of mixed Capsules contents taken shall be representative of the contents of the Capsules and shall, in turn, be weighed accurately.

6.70. Reagents

The proper conduct of the compendial procedures and the reliability of the results depend, in part, upon the quality of the reagents used in the performance of the procedures. Unless otherwise specified, reagents conforming to the specifications set forth in the current edition of *Reagent Chemicals* published by the American Chemical Society (ACS) shall be used. Where such ACS reagent specifications are not available or where the required purity differs, compendial specifications for reagents of acceptable quality are provided (see the *Reagents, Indicators, and Solutions* section of the USP-NF). Reagents not covered by any of these specifications should be of a grade suitable to the proper performance of the method of assay or test involved.

Listing of these reagents, including the indicators and solutions employed as reagents, in no way implies that they have therapeutic utility; furthermore, any reference to USP or NF in their labeling shall include also the term "reagent" or "reagent grade." USP may supply reagents if they otherwise may not be generally commercially available.

6.80. Equipment

Unless otherwise specified, a specification for a definite size or type of container or apparatus in a procedure is given solely as a recommendation. Other dimensions or types may be used if they are suitable for the intended use.

6.80.10. Apparatus for Measurement

Where volumetric flasks or other exact measuring, weighing, or sorting devices are specified, this or other equipment of at least equivalent accuracy shall be employed.

6.80.10.1. Pipet/Pipette

Where a pipet/pipette is specified, a suitable buret may be substituted. Where a "to contain" pipet/pipette is specified, a suitable volumetric flask may be substituted.

6.80.10.2. Light Protection

Where low-actinic or light-resistant containers are specified, either containers specially treated to protect contents from light or clear containers that have been rendered opaque by application of a suitable coating or wrapping may be used.

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6.80.20. Instrumental Apparatus

An instrument may be substituted for the specified instrument if the substitute uses the same fundamental principles of operation and is of equivalent or greater sensitivity and accuracy. These characteristics shall be qualified as appropriate. Where a particular brand or source of a material, instrument, or piece of equipment, or the name and address of a manufacturer or distributor, is mentioned (ordinarily in a footnote), this identification is furnished solely for informational purposes as a matter of convenience, without implication of approval, endorsement, or certification.

6.80.20.1. Chromatographic Tubes and Columns

The term "diameter" refers to internal diameter (ID).

6.80.20.2. Tubing

The term "diameter" refers to outside diameter (OD).

6.80.20.3. Steam Bath

Where use of a steam bath is directed, use actively flowing steam or another regulated heat source controlled at an equivalent temperature.

6.80.20.4. Water Bath

A water bath requires vigorously boiling water unless otherwise specified.

6.80.30. Temperature Reading Devices

Temperature reading devices suitable for pharmacopeial tests conform to specifications that are traceable to a National Institute of Standards and Technology (NIST) standard or equivalent. Temperature reading devices may be of the liquid-in-glass type or an analog or digital temperature indicator type, such as a resistance temperature device, thermistor, or thermocouple. Standardization of thermometers is performed on an established testing frequency with a temperature standard traceable to NIST. For example, refer to the current issue of American Society of Testing and Materials (ASTM) standards E1 for liquid-in-glass thermometers.

7. TEST RESULTS**7.10. Interpretation of Requirements**

Analytical results observed in the laboratory (or calculated from experimental measurements) are compared with stated acceptance criteria to determine whether the article conforms to compendial requirements.

The reportable value, which often is a summary value for several individual determinations, is compared with the acceptance criteria. The reportable value is the end result of a completed measurement procedure, as documented.

Where acceptance criteria are expressed numerically herein through specification of an upper and/or lower limit, permitted values include the specified values themselves, but no values outside the limit(s). Acceptance criteria are considered significant to the last digit shown.

7.10.5. Nominal Concentrations in Equations

Where a "nominal concentration" is specified, calculate the concentration based on the label claim. In assay procedures, water correction is typically stated in the Definition

and on the label of the USP Reference Standard. For other procedures, correction for assayed content, potency, or both is made prior to using the concentration in the equation provided in the monograph.

7.10.10. Equivalence Statements in Titrimetric Procedures

The directions for titrimetric procedures conclude with a statement of the weight of the analyte that is equivalent to each mL of the standardized titrant. In such an equivalence statement, the number of significant figures in the concentration of the titrant should be understood to correspond to the number of significant figures in the weight of the analyte. Corrections to calculations based on the blank determination are to be made for all titrimetric assays where appropriate (see *Titrimetry* (541)).

7.20. Rounding Rules

The observed or calculated values shall be rounded off to the number of decimal places that is in agreement with the limit expression. Numbers should not be rounded until the final calculations for the reportable value have been completed. Intermediate calculations (e.g., slope for linearity) may be rounded for reporting purposes, but the original (not rounded) value should be used for any additional required calculations. Acceptance criteria are fixed numbers and are not rounded.

When rounding is required, consider only one digit in the decimal place to the right of the last place in the limit expression. If this digit is smaller than 5, it is eliminated and the preceding digit is unchanged. If this digit is equal to or greater than 5, it is eliminated and the preceding digit is increased by 1.

Change to read:**8. TERMS AND DEFINITIONS****8.10. Abbreviations**

- RS refers to a USP Reference Standard.
- CS refers to a Colorimetric Solution.
- TS refers to a Test Solution.
- VS refers to a Volumetric Solution that is standardized in accordance with directions given in the individual monograph or in the *Reagents, Indicators, and Solutions* section of USP-NF.

8.20. About

"About" indicates a quantity within 10%.

If the measurement is stated to be "accurately measured" or "accurately weighed," follow the statements in *Volumetric Apparatus* (31) and *Balances* (41), respectively.

8.30. Alcohol Content

Percentages of alcohol, such as those under the heading *Alcohol Content*, refer to percentage by volume of C_2H_5OH at 15.56°. Where a formula, test, or assay calls for alcohol, ethyl alcohol, or ethanol, the USP monograph article Alcohol

**Illustration of Rounding Numerical Values
for Comparison with Requirements**

Compendial Requirement	Unrounded Value	Rounded Result	Conforms
Assay limit $\geq 98.0\%$	97.96%	98.0%	Yes
	97.92%	97.9%	No
	97.95%	98.0%	Yes
Assay limit $\leq 101.5\%$	101.55%	101.6%	No
	101.46%	101.5%	Yes
	101.45%	101.5%	Yes
Limit test $\leq 0.02\%$	0.025%	0.03%	No
	0.015%	0.02%	Yes
	0.027%	0.03%	No
Limit test ≤ 3 ppm	3.5 ppm	4 ppm	No
	3.4 ppm	3 ppm	Yes
	2.5 ppm	3 ppm	Yes

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shall be used. Where reference is made to " C_2H_5OH ," absolute (100 percent) ethanol is intended. Where a procedure calls for dehydrated alcohol, alcohol absolute, or anhydrous alcohol, the USP monograph article Dehydrated Alcohol shall be used.

8.40. Atomic Weights

Atomic weights used in computing molecular weights and the factors in the assays and elsewhere are those established by the IUPAC Commission on Isotopic Abundances and Atomic Weights. ▲ USP40

8.50. Blank Determinations

Where it is directed that "any necessary correction" be made by a blank determination, the determination shall be conducted using the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but with the substance itself omitted.

8.60. Concomitantly

"Concomitantly" denotes that the determinations or measurements are to be performed in immediate succession.

8.70. Desiccator

The instruction "in a desiccator" indicates use of a tightly closed container of suitable size and design that maintains an atmosphere of low moisture content by means of a suitable desiccant such as anhydrous calcium chloride, magnesium perchlorate, phosphorus pentoxide, or silica gel. See also section 8.220 *Vacuum Desiccator*.

8.80. Logarithms

Logarithms are to the base 10.

8.90. Microbial Strain

A microbial strain cited and identified by its American Type Culture Collection (ATCC) catalog number shall be used directly or, if subcultured, shall be used not more than five passages removed from the original strain.

8.100. Negligible

"Negligible" indicates a quantity not exceeding 0.50 mg.

8.110. NLT/NMT

"NLT" means "not less than." "NMT" means "not more than."

8.120. Odor

"Odorless," "practically odorless," "a faint characteristic odor," and variations thereof indicate evaluation of a suitable quantity of freshly opened material after exposure to the air for 15 minutes. An odor designation is descriptive only and should not be regarded as a standard of purity for a particular lot of an article.

8.130. Percent

"Percent" used without qualification means:

- For mixtures of solids and semisolids, percent weight in weight;
- For solutions or suspensions of solids in liquids, percent weight in volume;
- For solutions of liquids in liquids, percent volume in volume;
- For solutions of gases in liquids, percent weight in volume.

For example, a 1 percent solution is prepared by dissolving 1 g of a solid or semisolid, or 1 mL of a liquid, in sufficient solvent to make 100 mL of the solution.

8.140. Percentage Concentrations

Percentage concentrations are expressed as follows:

- **Percent Weight in Weight (w/w)** is defined as the number of g of a solute in 100 g of solution.
- **Percent Weight in Volume (w/v)** is defined as the number of g of a solute in 100 mL of solution.
- **Percent Volume in Volume (v/v)** is defined as the number of mL of a solute in 100 mL of solution.

8.150. Pressure

Pressure is determined by use of a suitable manometer or barometer calibrated in terms of the pressure exerted by a column of mercury of the stated height.

8.160. Reaction Time

Reaction time is 5 minutes unless otherwise specified.

8.170. Specific Gravity

Specific gravity is the weight of a substance in air at 25° divided by the weight of an equal volume of water at the same temperature.

8.180. Temperatures

Temperatures are expressed in centigrade (Celsius) degrees, and all measurements are made at 25° unless otherwise indicated. Where moderate heat is specified, any temperature not higher than 45° (113° F) is indicated.

8.190. Time

Unless otherwise specified, rounding rules, as described in section 7.20 *Rounding Rules*, apply to any time specified.

8.200. Transfer

"Transfer" indicates a quantitative manipulation.

8.210. Vacuum

"Vacuum" denotes exposure to a pressure of less than 20 mm of mercury (2.67 kPas), unless otherwise indicated.

8.220. Vacuum Desiccator

"Vacuum desiccator" indicates a desiccator that maintains a low-moisture atmosphere at a reduced pressure of not more than 20 mm of mercury (2.67 kPas) or at the pressure designated in the individual monograph.

8.230. Water**8.230.10. Water as an Ingredient in an Official Product**

As an ingredient in an official product, water meets the requirements of the appropriate water monograph in USP or NF.

8.230.20. Water in the Manufacture of Official Substances

When used in the manufacture of official substances, water shall meet the requirements for drinking water as set forth in the U.S. Environmental Protection Agency National Primary Drinking Water Regulations or in the drinking water regulations of the European Union or of Japan, or in the World Health Organization's Guidelines for Drinking Water Quality. Additional specifications may be required in monographs.

8.230.30. Water in a Compendial Procedure

When water is called for in a compendial procedure, the USP article Purified Water shall be used unless otherwise specified. Definitions for other types of water are provided in *Reagents, Indicators, and Solutions* and in *Water for Pharmaceutical Purposes* (1231).

8.240. Weights and Measures

In general, weights and measures are expressed in the International System of Units (SI) as established and revised by the *Conférence générale des poids et mesures*. For compendial purposes, the term "weight" is considered to be synonymous with "mass."

Molality is designated by the symbol *m* preceded by a number that represents the number of moles of the designated solute contained in 1 kilogram of the designated solvent.

Molarity is designated by the symbol *M* preceded by a number that represents the number of moles of the designated solute contained in an amount of the designated solvent that is sufficient to prepare 1 liter of solution.

Normality is designated by the symbol *N* preceded by a number that represents the number of equivalents of the designated solute contained in an amount of the designated solvent that is sufficient to prepare 1 liter of solution.

The symbol for degrees (°) without a qualifying unit of measure represents degrees Celsius.

Chart of Symbols and Prefixes commonly employed for SI metric units and other units:

	Units	Symbol	Notes
Length			
	meter	m	

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	Units	Symbol	Notes
	centimeter	cm	
	millimeter	mm	
	micrometer	μm	Previously referred to as a micron
	nanometer	nm	Previously the symbol $\text{m}\mu$ (for millimicron) was used
	Ångström	Å	Equal to 0.1 nm
Mass			
	kilogram	kg	
	gram	g	
	milligram	mg	
			The symbol μg is used in the USP and NF to represent micrograms, but micrograms may be represented as "mcg" for labeling and prescribing purposes. The term "gamma," symbolized by γ , frequently is used to represent micrograms in biochemical literature.
	microgram	μg	
	nanogram	ng	
	picogram	pg	
			Also referred to as the unified atomic mass unit and is equal to 1/12 times the mass of the free carbon 12 atom.
	dalton	Da	
	kilodalton	kDa	
Time			
	second	s	
	minute	min	
	hour	h	
Volume			
			1 L is equal to 1000 cm^3 (cubic centimeters)
	liter	L	
	deciliter	dL	
			1 mL is equal to 1 cm^3 , sometimes referred to as cc
	milliliter	mL	
	microliter	μL	
Temperature			
	Celsius	$^{\circ}\text{C}$	
Amount of Substance			
			Historically referred to as gram-molecular weight or gram-atomic weight
	mole	mol	
	millimole	mmol	
	micromole	μmol	
	femtomole	fmol	

	Units	Symbol	Notes
			Also referred to as gram-equivalent weight. It is used in the calculation of substance concentration in units of normality. This unit is no longer preferred for use in analytical chemistry or metrology.
	equivalent	Eq	
	milli equivalent	mEq	
			Osmotic pressure of a solution, related to substance concentration.
	osmole	Osmol	
	milliosmole	mOsmol	
Pressure			
	pascal	Pa	
	kilopascal	kPa	
	pounds per square inch	psi	
	millimeter of mercury	mmHg	Equal to 133.322 Pa
Electrical units			
	ampere	A	
	volt	V	
	millivolt	mV	
	hertz	Hz	Unit of frequency
	kilohertz	kHz	
	megahertz	MHz	
	electron volt	eV	
	kilo-electron volt	keV	
	mega-electron volt	MeV	
Radiation			
	becquerel	Bq	SI unit of activity for radionuclides
	kilobecquerel	kBq	
	megabecquerel	MBq	
	gigabecquerel	GBq	
	curie	Ci	Non-SI unit of activity for radionuclides
	millicurie	mCi	
	microcurie	μCi	
	nanocurie	nCi	
Other			
	acceleration due to gravity	g	Used to express rate of centrifugation
	revolutions per minute	rpm	Used to express rate of centrifugation

Selected SI Prefixes

Name	Symbol	Factor
giga	G	10^9
mega	M	10^6

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Selected SI Prefixes (Continued)

Name	Symbol	Factor
kilo	k	10^3
deci	d	10^{-1}
centi	c	10^{-2}
milli	m	10^{-3}
micro	μ	10^{-6}
nano	n	10^{-9}
pico	p	10^{-12}
femto	f	10^{-15}

9. PRESCRIBING AND DISPENSING**9.10. Use of Metric Units**

Prescriptions for compendial articles shall be written to state the quantity and/or strength desired in metric units unless otherwise indicated in the individual monograph (see also section 5.50.10 *Units of Potency [Biological]* above). If an amount is prescribed by any other system of measurement, only an amount that is the metric equivalent of the pre-

scribed amount shall be dispensed. Abbreviations for the terms "Units" or "International Units" shall not be used for labeling or prescribing purposes. Apothecary unit designations on labels and labeling shall not be used.

9.20. Changes in Volume

In the dispensing of prescription medications, slight changes in volume owing to variations in room temperatures may be disregarded.

10. PRESERVATION, PACKAGING, STORAGE, AND LABELING**10.10. Packaging and Storage**

All articles in *USP* or *NF* are subject to the packaging and storage requirements specified in *Packaging and Storage Requirements* (659), unless different requirements are provided in an individual monograph.

10.20. Labeling

All articles in *USP* or *NF* are subject to the labeling requirements specified in *Labeling* (7), unless different requirements are provided in an individual monograph.

Juniper Tar

DEFINITION

Juniper Tar is the empyreumatic volatile oil obtained from the woody portions of *Juniperus oxycedrus* L. (Fam. Pinaceae).

IDENTIFICATION

- **A.**
Sample solution: Shake 1 volume of Juniper Tar with 20 volumes of warm water, filter, and use the filtrate.
Analysis: To 5 mL of the cold *Sample solution* add a few drops of silver-ammonium nitrate TS.
Acceptance criteria: A black color is produced.
- **B.**
Sample solution: Shake 1 volume of Juniper Tar with 20 volumes of warm water, filter, and use the filtrate.
Analysis: To 5 mL of the *Sample solution* add a few drops of alkaline cupric tartrate TS, and heat the solution to boiling.
Acceptance criteria: A red precipitate is formed.

SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 0.950–1.055
- **REACTION:** The filtrate prepared for *Identification* test A is acid to litmus.

ROSIN OR ROSIN OILS

Sample solution: Triturate 1 mL of Juniper Tar with 15 mL of solvent hexane, and filter.

Analysis: Mix equal volumes of *Sample solution* and cupric acetate solution (10 mg/mL), shake vigorously, and allow the liquids to separate. Decant the solvent hexane layer into a test tube, and add an equal volume of ether.

Acceptance criteria: The liquid does not become dark green or blackish.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

Kanamycin Injection

DEFINITION

Kanamycin Injection contains an amount of kanamycin sulfate equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of kanamycin ($C_{18}H_{36}N_4O_{11}$). It contains suitable buffers and preservatives.

IDENTIFICATION

A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

Sample solution: 1 mg/mL of kanamycin from Injection in water

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, heated at 110° for 1 h and cooled immediately before use

Application volume: 10 μ L

Developing solvent system: 150 mg/mL of monobasic potassium phosphate in water

Spray reagent: 10 mg/mL of ninhydrin in butyl alcohol

Analysis: Proceed as directed in the chapter. Allow the spots to dry, and develop in a chamber previously equilibrated for 18 h with the *Developing solvent system*. Remove the plate from the chamber, and air-dry. Spray the plate with *Spray reagent*, and dry at 110° for 10 min.

Acceptance criteria: Meets the requirements

B. The retention time of the kanamycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Mobile phase: 0.115 N sodium hydroxide solution
System suitability solution: 20 μ g/mL of USP Amikacin RS and 8 μ g/mL of USP Kanamycin Sulfate RS in water

Standard solution: 8 μ g/mL of USP Kanamycin Sulfate RS in water

Sample solution: Nominally 6 μ g/mL of kanamycin from Injection in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Electrochemical

Mode: Integrated amperometric

Range: 300 nC

Output: 1 V full-scale

Electrodes

Indicator: Gold

Reference: pH silver-silver chloride

Waveform: See *Table 1*.

Table 1

Time (s)	Potential (V)	Integration
0.00	+0.04	—
0.30	+0.04	Begin
0.50	+0.04	End
0.51	+0.80	—
0.70	+0.80	—
0.71	-0.80	—
0.90	-0.80	—

Columns

Guard: Packing L47

Analytical: 4-mm \times 25-cm; packing L47

Flow rate: 0.5 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for kanamycin and amikacin are about 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 3 between kanamycin and amikacin, *System suitability solution*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of kanamycin ($C_{18}H_{36}N_4O_{11}$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Kanamycin Sulfate RS in the *Standard solution* (μ g/mL)

C_U = nominal concentration of kanamycin in the *Sample solution* (μ g/mL)

P = potency of kanamycin in USP Kanamycin Sulfate RS (μ g/mg)

F = conversion factor, 0.001 mg/ μ g

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

• **pH (791):** 3.5–5.0

• **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.67 USP Endotoxin Unit/mg of kanamycin

• **STERILITY TESTS (71):** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass.

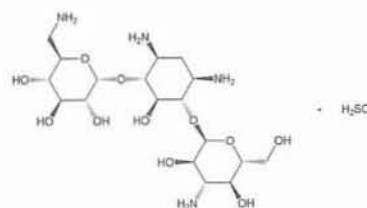
• **USP REFERENCE STANDARDS (11)**

USP Amikacin RS

USP Endotoxin RS

USP Kanamycin Sulfate RS

Kanamycin Sulfate



582.58

D-Streptamine, O-3-amino-3-deoxy- α -D-glucopyranosyl(1 \rightarrow 6)-O-[6-amino-6-deoxy- α -D-glucopyranosyl(1 \rightarrow 4)]-2-deoxy-, sulfate (1:1) (salt); Kanamycin sulfate (1:1) (salt) [25389-94-0].

DEFINITION

Kanamycin Sulfate has a potency equivalent to NLT 750 μ g/mg of kanamycin ($C_{18}H_{36}N_4O_{11}$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL**, Sulfate (191): Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Mobile phase: 0.115 N sodium hydroxide solution
System suitability solution: 20 μ g/mL of USP Amikacin RS and 8 μ g/mL of USP Kanamycin Sulfate RS in water
Standard solution: 8 μ g/mL of USP Kanamycin Sulfate RS in water

Sample solution: 8 μ g/mL of Kanamycin Sulfate in water

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: Pulsed amperometric electrochemical detector

Working electrode: Gold

Reference electrode: pH silver–silver chloride

Waveform: See Table 1.

Table 1

Time (s)	Potential (V)	Integration
0.00	+0.04	—
0.30	+0.04	Begin
0.50	+0.04	End
0.51	+0.80	—
0.70	+0.80	—
0.71	−0.80	—
0.90	−0.80	—

Columns

Guard: 4-mm \times 50-mm; 7.5- μ m packing L47

Analytical: 4-mm \times 25-cm; 7.5- μ m packing L47

Flow rate: 0.5 mL/min

Injection volume: 20 μ L

System suitability

[NOTE—The relative retention times for kanamycin and amikacin are about 1.0 and 1.3, respectively.]

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3 between kanamycin and amikacin, *System suitability solution*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
 Calculate the quantity, in μ g/mg, of kanamycin ($C_{18}H_{36}N_4O_{11}$) in the portion of Kanamycin Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

- r_U = peak area from the *Sample solution*
- r_S = peak area from the *Standard solution*
- C_S = concentration of USP Kanamycin Sulfate RS in the *Standard solution* (μ g/mL)
- C_U = concentration of Kanamycin Sulfate in the *Sample solution* (μ g/mL)
- P = potency of kanamycin in USP Kanamycin Sulfate RS (μ g/mg)

Acceptance criteria: NLT 750 μ g/mg on the dried basis

IMPURITIES

• RESIDUE ON IGNITION (281)

Analysis: Moisten the charred residue with 2 mL of nitric acid and 5 drops of sulfuric acid.

Acceptance criteria: NMT 1.0%

• ORGANIC IMPURITIES

Adsorbent: 0.25-mm layer of chromatographic silica gel

Developing solvent system: 75 mg/mL of monobasic potassium phosphate in water

Spray reagent: 10 mg/mL of ninhydrin in butyl alcohol

Standard solution 1: 30 mg/mL of USP Kanamycin Sulfate RS in water

Standard solution 2: 0.90 mg/mL of USP Kanamycin Sulfate RS in water

Sample solution: 30 mg/mL of Kanamycin Sulfate in water

Application volume: 1 μ L

Analysis: Heat the plate at 110° for 1 h immediately before use, and allow it to cool. Equilibrate for 90 min with the *Developing solvent system*.

Apply all three solutions to the plate separately, allow the spots to dry, and develop the chromatogram with the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, and air-dry.

Spray the plate with *Spray reagent*. Dry the plate at 110° for 10 min, and examine the chromatograms.

Acceptance criteria: The chromatograms show principal spots at the same R_f value, and no secondary spot, if present from the *Sample solution*, is more intense than the principal spot of *Standard solution 2*.

SPECIFIC TESTS

- **CRYSTALLINITY** (695): Meets the requirements

• pH (791)

Sample solution: 10 mg/mL

Acceptance criteria: 6.5–8.5

• LOSS ON DRYING (731)

Analysis: Dry 100 mg in a vacuum in a capillary-stoppered bottle at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

Acceptance criteria: NMT 4.0%

- **STERILITY TESTS** (71): Where the label states that Kanamycin Sulfate is sterile, it meets the requirements when tested as directed for membrane filtration in *Test for Sterility of the Product to Be Examined*.

- **BACTERIAL ENDOTOXINS TEST** (85): Where the label states that Kanamycin Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.67 USP Endotoxin Unit/mg of Kanamycin.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP REFERENCE STANDARDS** (11)

USP Amikacin RS
USP Endotoxin RS
USP Kanamycin Sulfate RS

Kanamycin Sulfate Capsules

DEFINITION

Kanamycin Sulfate Capsules contain the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of kanamycin ($C_{18}H_{36}N_4O_{11}$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

Sample solution: 1 mg/mL of kanamycin from Capsules in water

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, heated at 110° for 1 h and cooled immediately before use

Application volume: 10 μ L

Developing solvent system: 150 mg/mL of monobasic potassium phosphate solution

Spray reagent: 10 mg/mL of ninhydrin in butyl alcohol

Analysis: Proceed as directed in the chapter. Allow the spots to dry, and develop in a suitable chamber, previously equilibrated for 18 h with the *Developing solvent system*. Remove the plate from the chamber, and air-dry. Spray the plate with *Spray reagent*, and dry at 110° for 10 min.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the kanamycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: 0.115 N sodium hydroxide solution

System suitability solution: 20 μ g/mL of USP Amikacin RS and 8 μ g/mL of USP Kanamycin Sulfate RS

Standard solution: 8 μ g/mL of USP Kanamycin Sulfate RS

Sample stock solution: Nominally 0.32 mg/mL of kanamycin, prepared as follows. Transfer a portion of the mixed contents of Capsules (NLT 10) to a suitable volumetric flask. Add water, using 20% of the final volume, and swirl to dissolve. Dilute with water to volume.

Sample solution: Nominally 6.4 μ g/mL of kanamycin from the *Sample stock solution* in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Electrochemical

Mode: Integrated amperometric

Range: 300 nC

Output: 1 V full-scale

Electrodes

Indicator: Gold

Reference: pH silver-silver chloride

Waveform: See *Table 1*.

Table 1

Time (s)	Potential (V)	Integration
0.00	+0.04	—
0.30	+0.04	Begin

Table 1 (Continued)

Time (s)	Potential (V)	Integration
0.50	+0.04	End
0.51	+0.80	—
0.70	+0.80	—
0.71	−0.80	—
0.90	−0.80	—

Columns

Guard: Packing L47

Analytical: 4-mm \times 25-cm; packing L47

Flow rate: 0.5 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for kanamycin and amikacin are about 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 3 between kanamycin and amikacin, *System suitability solution*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of kanamycin ($C_{18}H_{36}N_4O_{11}$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Kanamycin Sulfate RS in the *Standard solution* (μ g/mL)

C_U = nominal concentration of kanamycin in the *Sample solution* (μ g/mL)

P = potency of kanamycin in USP Kanamycin Sulfate RS (μ g/mg)

F = conversion factor, 0.001 mg/ μ g

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

- **DISSOLUTION, Procedure for a Pooled Sample** (711)

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: USP Kanamycin Sulfate RS in *Medium*

Sample solution: Sample per *Dissolution* (711). Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Analysis: Determine the percentage of the labeled amount of kanamycin ($C_{18}H_{36}N_4O_{11}$) dissolved by using the procedure described in the *Assay*, making any necessary modifications.

Tolerances: NLT 75% (Q) of the labeled amount of kanamycin ($C_{18}H_{36}N_4O_{11}$) is dissolved.

SPECIFIC TESTS

- **LOSS ON DRYING** (731)

Sample: 100 mg

Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.

Acceptance criteria: NMT 4.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Amikacin RS
USP Kanamycin Sulfate RS

Kaolin

DEFINITION

Kaolin is a native hydrated aluminum silicate, powdered and freed from gritty particles by elutriation.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Aluminum (191)**

Sample solution: Mix 1 g of Kaolin with 10 mL of water and 5 mL of sulfuric acid in a porcelain dish. Evaporate the mixture until the excess water is removed, and continue heating the residue until dense, white fumes of sulfur trioxide appear. Cool, cautiously add 20 mL of water, boil for a few min, and filter.

Acceptance criteria: A gray residue (impure silica) remains on the filter, and the filtrate meets the requirements of the test.

IMPURITIES

- **LOSS ON IGNITION (733)**

Analysis: Ignite between 550° and 600°.

Acceptance criteria: NMT 15.0%

- **LEAD (251)**

Test preparation: Transfer 1.0 g of Kaolin to a centrifuge tube, add 10 mL of 1 N nitric acid, and digest for 1 h in a boiling water bath. Centrifuge until the solids are completely separated, and pour the supernatant into a 100-mL volumetric flask. Add 5 mL of 1 N nitric acid, and digest for 15 min in a boiling water bath. Centrifuge, and add the supernatant to the previous extract in the volumetric flask. Dilute with water to volume.

Analysis: Proceed as directed in the chapter, using a 50-mL portion of this solution, 3 mL of *Ammonium Citrate Solution*, 500 µL of *Hydroxylamine Hydrochloride Solution*, and 1 mL of *Potassium Cyanide Solution*.

Acceptance criteria: NMT 5 µg of lead (NMT 10 ppm)

- **IRON**

Sample: 2.0 g

Analysis: Triturate the *Sample* in a mortar with 10 mL of water, and add 0.50 g of sodium salicylate.

Acceptance criteria: The mixture does not acquire more than a slight reddish tint.

- **ACID-SOLUBLE SUBSTANCES**

Sample: 1.0 g

Analysis: Digest the *Sample* with 20 mL of 3 N hydrochloric acid for 15 min, and filter.

Acceptance criteria: NMT 2.0%; 10 mL of the filtrate, evaporated to dryness and ignited, leaves NMT 10 mg of residue.

- **CARBONATE**

Sample: 1.0 g

Analysis: Mix the *Sample* with 10 mL of water and 5 mL of sulfuric acid.

Acceptance criteria: No effervescence occurs.

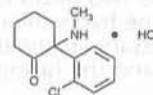
SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the tests for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Ketamine Hydrochloride



$C_{13}H_{16}ClNO \cdot HCl$ 274.19

Cyclohexanone, 2-(2-chlorophenyl)-2-(methylamino)-, hydrochloride.

(±)-2-(o-Chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride [1867-66-9].

» Ketamine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{13}H_{16}ClNO \cdot HCl$.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards (11)—

USP Ketamine Hydrochloride RS

USP Ketamine Related Compound A RS

1-[(2-Chlorophenyl)(methylimino)methyl]cyclopentanol.
 $C_{13}H_{16}NOCl$ 237.73

Clarity and color of solution—Dissolve 1 g in 5 mL of water; the solution is clear and colorless.

Identification—

A: Infrared Absorption (197K)—Do not dry specimens.

B: Acid solvent—The UV absorption spectrum of a solution in 0.1 N hydrochloric acid (1 in 3000) exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Ketamine Hydrochloride RS, concomitantly measured, and the respective absorptivities, at the wavelengths of maximum absorbance at about 269 and 276 nm, do not differ by more than 3.0%.

Basic solvent—The UV absorption spectrum of a solution in 0.01 N sodium hydroxide (1 in 1250), in a mixture of water and methanol (1 in 20), exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Ketamine Hydrochloride RS, concomitantly measured, and the respective absorptivities, at the wavelength of maximum absorbance at about 302 nm, do not differ by more than 3.0%.

pH (791): between 3.5 and 4.1, in a solution (1 in 10).

Residue on ignition (281): not more than 0.1%.

Delete the following:

- **Heavy metals, Method I (231):** 0.002%. • (Official 1-Jan-2018)

Related compounds—

Mobile phase—Dissolve 0.95 g of sodium 1-hexanesulfonate in 1 L of a solution consisting of a mixture of water and acetonitrile (3:1). Add 4 mL of acetic acid, and mix.

Standard solution—Dissolve accurately weighed quantities of USP Ketamine Hydrochloride RS and USP Ketamine Related Compound A RS in *Mobile phase* (sonicate if necessary) to prepare a solution containing about 0.005 mg per mL of each compound. Prepare immediately before use.

Test solution—Transfer an accurately weighed quantity of about 50.0 mg of Ketamine Hydrochloride to a 50-mL volumetric flask. Dissolve in and dilute with *Mobile phase* to volume, sonicating if necessary.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.0-mm × 4.0-mm guard column with a 4.0-mm × 12.5-cm analytical column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph

the *Standard solution*, and record the peak responses as directed for *Procedure*: the order of elution is ketamine hydrochloride followed by ketamine related compound A; the resolution, R , between these two peaks is not less than 2.0; the retention time of ketamine hydrochloride is between 3.0 and 4.5 minutes (if necessary, adjust the concentration of water and acetonitrile); and the tailing factor is not greater than 1.5.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, identify the ketamine hydrochloride and ketamine related compound A peaks, and measure the areas of the major peaks. Calculate the area percentage of each impurity, relative to ketamine hydrochloride, in the portion of Ketamine Hydrochloride taken by the formula:

$$5000(C/W)(r_i / r_s)$$

in which C is the concentration, in mg per mL, of USP Ketamine Hydrochloride RS in the *Standard solution*; W is the weight, in mg, of Ketamine Hydrochloride taken to prepare the *Test solution*; r_i is the peak area of each individual impurity peak in the *Test solution*; and r_s is the response of the ketamine hydrochloride peak obtained from the *Standard solution*. Not more than 0.1% of ketamine related compound A is found; the response of no other unknown impurity is greater than 0.3% of the ketamine peak area; and the sum of the responses of all unknown impurity peaks is not greater than 1.0% of the ketamine peak response.

Assay—

Buffer—Dissolve 5.75 g of monobasic ammonium phosphate in 1000 mL of water. Add 6 mL of triethylamine, and adjust with phosphoric acid to a pH of 3.0.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer* and methanol (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability solution—Transfer about 12.5 mg each, of USP Ketamine Hydrochloride RS and USP Ketamine Related Compound A RS, both accurately weighed, to a 50-mL volumetric flask, dissolve in *Mobile phase* with the aid of sonification if necessary, dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of the solution so obtained to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Standard preparation—Transfer about 10 mg of USP Ketamine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add about 20 mL of *Mobile phase*, and sonicate to dissolve. Dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer about 20 mg of Ketamine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, add about 35 mL of *Mobile phase*, and sonicate to dissolve. Dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm \times 25-cm column that contains 5- μ m packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the order of elution is ketamine followed by ketamine related compound A; the resolution, R , between ketamine and ketamine related compound A is not less than 2.0; the column efficiency determined from the ketamine peak is not less than 9400 theoretical plates; and the tailing factor determined from the ketamine peak is not more than 1.6. Chromatograph the *Standard preparation*, and record the ketamine peak response as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 0.6%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into

the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{13}H_{16}ClNO \cdot HCl$ in the portion of Ketamine Hydrochloride taken by the formula:

$$100C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Ketamine Hydrochloride RS in the *Standard preparation*; and r_u and r_s are the ketamine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ketamine Hydrochloride Injection

» Ketamine Hydrochloride Injection is a sterile solution of Ketamine Hydrochloride in Water for Injection. It contains an amount of ketamine hydrochloride ($C_{13}H_{16}ClNO \cdot HCl$) equivalent to not less than 95.0 percent and not more than 105.0 percent of the labeled amount of ketamine ($C_{13}H_{16}ClNO$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light and heat.

USP Reference standards (11)—

USP Endotoxin RS

USP Ketamine Hydrochloride RS

Identification—

A: The UV absorption spectrum, measured in the region between 250 and 350 nm, of a dilution of Injection in 0.01 N methanolic sodium hydroxide containing ketamine hydrochloride equivalent to about 800 μ g of ketamine per mL, exhibits maxima and minima at the same wavelengths as that of a similar preparation of USP Ketamine Hydrochloride RS, concomitantly measured.

B: The UV absorption spectrum of the solution employed for measurement of absorbance of the assay solution, prepared as directed in the *Assay*, exhibits maxima and minima at the same wavelengths as that of the *Standard solution*, prepared as directed in the *Assay*.

Bacterial Endotoxins Test (85)—It contains not more than 0.4 USP Endotoxin Unit per mg of ketamine hydrochloride.

pH (791): between 3.5 and 5.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

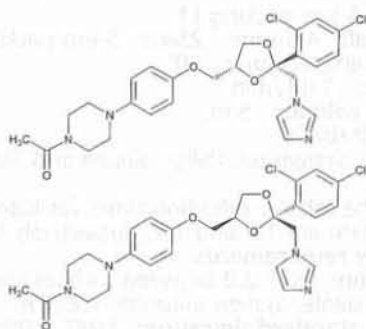
Assay—Transfer an accurately measured volume of Injection, equivalent to about 500 mg of ketamine hydrochloride, to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 20.0 mL of this solution to a 125-mL separator, add 3 mL of 0.1 N sodium hydroxide, and extract with three 15-mL portions of chloroform. Collect the chloroform extracts in a second 125-mL separator, and extract with three 30-mL portions of 0.1 N sulfuric acid, collecting the acid extracts in a 200-mL volumetric flask. Dilute with 0.1 N sulfuric acid (saturated with chloroform) to volume, and mix. Concomitantly determine the absorbances of this solution and a *Standard solution* of USP Ketamine Hydrochloride RS in the same medium having a known concentration of about 250 μ g per mL, in 1-cm cells at the wavelength of maximum absorbance at about 269 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid (saturated with chloroform) as the blank. Calculate the quantity,

in mg, of ketamine ($C_{13}H_{16}ClNO$) in each mL of the Injection taken by the formula:

$$(237.73 / 274.19)(2C / V)(A_U / A_S)$$

in which 237.73 and 274.19 are the molecular weights of ketamine and ketamine hydrochloride, respectively; C is the concentration, in μg per mL, of USP Ketamine Hydrochloride RS in the Standard solution; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Ketoconazole



$C_{26}H_{28}Cl_2N_4O_4$ 531.43
Piperazine, 1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-, *cis*-;
(±)-*cis*-1-Acetyl-4-(p-[[2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl)piperazine [65277-42-1].

DEFINITION

Ketoconazole contains NLT 98.0% and NMT 102.0% of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Buffer: 3.4 mg/mL of tetrabutyl ammonium hydrogen sulfate in water
Solution A: Acetonitrile and Buffer (5:95)
Solution B: Acetonitrile and Buffer (50:50)
Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	0	100
25	0	100
26	100	0
30	100	0

Diluent: Methanol

Standard solution: 0.1 mg/mL of USP Ketoconazole RS in Diluent

Sample solution: 0.1 mg/mL of Ketoconazole in Diluent

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 10-cm; 3- μm packing L1

Flow rate: 2.0 mL/min

Injection volume: 10 μL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of ketoconazole

($C_{26}H_{28}Cl_2N_4O_4$) in the portion of Ketoconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ketoconazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Ketoconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• RESIDUE ON IGNITION (281)

Sample: 2 g

Acceptance criteria: NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-

Jan-2018)

• ORGANIC IMPURITIES

Buffer, Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 0.01 mg/mL each of USP

Ketoconazole RS and USP Terconazole RS in Diluent

Sample solution: 10.0 mg/mL of Ketoconazole in Diluent

System suitability

Sample: Standard solution

Suitability requirements

Resolution: NLT 2.0 between ketoconazole and terconazole peaks

Relative standard deviation: NMT 5.0% for the ketoconazole peak

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of any impurities in the portion of Ketoconazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any impurities from the *Sample solution*

r_S = peak response of Ketoconazole from the *Standard solution*

C_S = concentration of USP Ketoconazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Ketoconazole in the *Sample solution* (mg/mL)

Acceptance criteria: Disregard any peak less than 0.05%.

Any individual unspecified impurity: NMT 0.10%
Total impurities: NMT 2.0%

SPECIFIC TESTS• **OPTICAL ROTATION, Specific Rotation (7815)**

Sample solution: 40 mg/mL in methanol

Acceptance criteria: -1° to $+1^{\circ}$ at 20°

• **LOSS ON DRYING (731)**

Analysis: Dry under vacuum at 80° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in well-closed containers.• **USP REFERENCE STANDARDS (11)**

USP Ketoconazole RS

USP Terconazole RS

Piperazine, 1-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-4-(1-methylethyl)-, *cis*;

cis-1-(p-[[2-(2,4-Dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl)-4-isopropylpiperazine.

$C_{26}H_{31}Cl_2N_5O_3$ 532.46

Ketoconazole Compounded Oral Suspension

DEFINITION

Ketoconazole Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$).

Prepare Ketoconazole Compounded Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Ketoconazole	2.0 g
Cetylpyridinium Chloride	10 mg
Xanthan Gum	0.15 g
Purified Water	30 mL
Suspension Structured Vehicle or Sugar-Free Suspension Structured Vehicle, a sufficient quantity to make	100 mL

Place the required number of tablets in a glass mortar, and comminute to a fine powder such that they pass through a 40-mesh or 45-mesh sieve, or add Ketoconazole powder to the mortar. Dissolve the Cetylpyridinium Chloride in Purified Water, and dilute quantitatively, and stepwise if necessary, with Purified Water to obtain 10 mL of a solution containing 10 mg of Cetylpyridinium Chloride. Transfer this solution, in divided portions, to the mortar containing the powder, and mix to form a smooth paste. Place 20 mL of Purified Water in a beaker. Using moderate heat, stir to form a vortex, and slowly sprinkle the Xanthan Gum into the vortex to obtain a uniform dispersion. Add the dispersion to the wetted powder paste, and mix until smooth. Add a sufficient quantity of the Suspension Structured Vehicle or Sugar-Free Suspension Structured Vehicle to bring to final volume. Mix well.

ASSAY• **PROCEDURE**

Mobile phase: Acetonitrile and 0.01 M tetrabutylammonium hydrogen sulfate (25:75). Pass through a filter of 5- μ m or finer pore size, and degas.

Diluent: Methanol and water (50:50)

System suitability solution: Dissolve 4 mg of USP Ketoconazole RS in 1.0 mL of a solution of potassium sorbate in water (1 in 5000). Dilute with Diluent to 10.0 mL.

Standard solution: 0.4 mg/mL of USP Ketoconazole RS in Diluent

Sample solution: [NOTE—If the Oral Suspension has settled, invert the container 10–15 times, and sonicate for 5 min, or stir on a magnetic stirrer until the Oral Suspension appears homogeneous. Examine the mixture for the presence of bubbles and unsuspended solids before sampling.] Transfer 5.0 mL of homogeneous Oral Suspension to a 250-mL volumetric flask, add 100 mL of water, and stir for 15 min to dissolve the xanthan gum. Add 135 mL of methanol, and stir for an additional 15 min. Cool, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 223 nm

Columns

Guard: 5- μ m packing L1

Analytical: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 5 μ L

System suitability

Samples: System suitability solution and Standard solution

[NOTE—The relative retention times for ketoconazole and sorbate are 1.0 and 1.7, respectively.]

Suitability requirements

Resolution: NLT 2.0 between sorbate and ketoconazole, System suitability solution

Relative standard deviation: NMT 2.0% for replicate injections, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Ketoconazole RS in the Standard solution (mg/mL)

C_U = nominal concentration of ketoconazole in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.• **BEYOND-USE DATE:** NMT 14 days after the date on which it was compounded when stored at controlled room temperature• **LABELING:** Label it to state that it is to be well shaken before use, and that it is to be protected from light. Label it to state the Beyond-Use Date.• **USP REFERENCE STANDARDS (11)**

USP Ketoconazole RS

Ketoconazole Tablets

DEFINITION

Ketoconazole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$).

IDENTIFICATION• **A.**

Standard solution: 1 mg/mL of USP Ketoconazole RS in chloroform

Sample solution: Nominally 1 mg/mL of ketoconazole in chloroform prepared as follows. Transfer a quantity of finely powdered Tablets, equivalent to 50 mg of ketoconazole, to a suitable flask. Add 50 mL of chloroform, shake for 2 min, and filter.

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: *n*-Hexane, ethyl acetate, methanol, glacial acetic acid, and water (42:40:15:1:2)

Analysis: Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and view under short-wavelength UV light.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• PROCEDURE

Solution A: Diisopropylamine in methanol (1 in 500)

Solution B: 5 mg/mL of ammonium acetate in water

Mobile phase: *Solution A* and *Solution B* (7:3)

Diluent: Methanol and methylene chloride (1:1)

Internal standard solution: 5 mg/mL of USP Terconazole RS in *Diluent*

Standard solution: 0.4 mg/mL of USP Ketoconazole RS in *Diluent* prepared as follows. Transfer 20 mg of USP Ketoconazole RS to a 50-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with *Diluent*.

Sample stock solution: Nominally 4 mg/mL of ketoconazole in *Diluent* prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer the nominal equivalent to 200 mg of ketoconazole to a suitable screw-capped bottle. Add 50.0 mL of *Diluent*, shake by mechanical means for 30 min, and centrifuge.

Sample solution: Nominally 0.4 mg/mL of ketoconazole in *Diluent* prepared as follows. Transfer 5.0 mL of the clear supernatant so obtained from the *Sample stock solution* to a 50-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 225 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 3 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ketoconazole and terconazole are 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between ketoconazole and terconazole

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of ketoconazole to terconazole from the *Sample solution*

R_S = peak response ratio of ketoconazole to terconazole from the *Standard solution*

C_S = concentration of USP Ketoconazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ketoconazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Analytical wavelength: UV 270 nm

Standard solution: USP Ketoconazole RS in *Medium*

Sample solutions: Pass portions of the solution under test through a suitable filter of 0.45- μ m pore size, and dilute with *Medium* to a concentration similar to that of the *Standard solution*.

Tolerances: NLT 80% (Q) of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

ADDITIONAL REQUIREMENTS

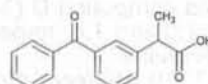
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• USP REFERENCE STANDARDS (11)

USP Ketoconazole RS

USP Terconazole RS

Ketoprofen



$C_{16}H_{14}O_3$

Benzeneacetic acid, 3-benzoyl- α -methyl-, (\pm); (\pm)-*m*-Benzoylhydratropic acid [22071-15-4].

254.28

DEFINITION

Ketoprofen contains NLT 98.5% and NMT 101.0% of $C_{16}H_{14}O_3$, calculated on the dried basis.

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

• B. ULTRAVIOLET ABSORPTION (197U)

Analytical wavelength: 258 nm

Medium: Methanol and water (3:1)

Blank: *Medium*

Sample solution: 10 μ g/mL in *Medium*

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY

• PROCEDURE

Sample: 450 mg

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N sodium hydroxide VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 25 mL of alcohol. Add 25 mL of water and several drops of phenol red TS.

Perform a blank determination. Each mL of *Titrant* is equivalent to 25.43 mg of $C_{16}H_{14}O_3$. [NOTE—Standardize the 0.1 N sodium hydroxide by a similar titration of primary standard benzoic acid.]

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

Delete the following:

- **HEAVY METALS**, Method II (231): NMT 0.002% (Official 1: Jan-2018)

ORGANIC IMPURITIES

[NOTE—Protect the solutions from light.]

Buffer: 68 g/L of monobasic potassium phosphate in water, and adjust with phosphoric acid to a pH of 3.5 ± 0.05

Mobile phase: Acetonitrile, water, and Buffer (43:55:2)

System suitability solution: 5 µg/mL of USP

Ketoprofen RS and 1.5 µg/mL of USP Ketoprofen Related Compound D RS in *Mobile phase*

Standard solution: 0.002 mg/mL of USP Ketoprofen RS in *Mobile phase*

Sample solution: 1 mg/mL of Ketoprofen in *Mobile phase*

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 233 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

Run time: Seven times the retention time for Ketoprofen

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for ketoprofen and ketoprofen related compound D (3-acetylbenzophenone) are about 1.0 and 1.6, respectively.]

Suitability requirements

Resolution: NLT 7.0 between ketoprofen related compound D and ketoprofen, *System suitability solution*

Column efficiency: NLT 2250 theoretical plates from ketoprofen, *System suitability solution*

Tailing factor: NMT 2.0 for ketoprofen, *System suitability solution*

Relative standard deviation: NMT 5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ketoprofen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of impurity from the *Sample solution*

r_S = peak response of ketoprofen from the *Standard solution*

C_S = concentration of USP Ketoprofen RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria

Any individual impurity: NMT 0.2%

Total impurities: NMT 1.0%

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE**, Procedure for Class I (741): 92.0°–97.0°

- **OPTICAL ROTATION**, Specific Rotation (781S)

Sample solution: 10 mg/mL, in dehydrated alcohol

Acceptance criteria: +1° to –1°

- **LOSS ON DRYING** (731): Dry a sample in a vacuum at 60° for 4 h; it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)
 - USP Ketoprofen RS
 - USP Ketoprofen Related Compound D RS
 - 3-Acetylbenzophenone.

Ketoprofen Capsules

DEFINITION

Ketoprofen Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of ketoprofen ($C_{16}H_{14}O_3$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

Sample solution: Shake a quantity of the contents of the Capsules containing 50 mg of ketoprofen with 5 mL of chloroform for 5 min, filter, and evaporate to dryness using a rotary evaporator.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

- **PROCEDURE**

The *Standard solution* and *Sample solution* must be protected from light.

Mobile phase: Acetonitrile, glacial acetic acid, and water (90:1:10)

System suitability stock solution: 0.25 mg/mL of USP Ketoprofen RS and 0.5 mg/mL of USP Ketoprofen Related Compound A RS in *Mobile phase*

System suitability solution: 0.02 mg/mL of USP Ketoprofen RS and 0.04 mg/mL of USP Ketoprofen Related Compound A RS in *Mobile phase* from *System suitability stock solution*

Standard stock solution: 0.24 mg/mL of USP Ketoprofen RS in *Mobile phase*

Standard solution: 0.024 mg/mL of USP Ketoprofen RS in *Mobile phase* from *Standard stock solution*

Sample solution: Nominally 0.024 mg/mL of ketoprofen in *Mobile phase* prepared as follows. Remove completely the contents of NLT 20 Capsules, and transfer a quantity of the contents, equivalent to 200 mg of ketoprofen, to a 250-mL volumetric flask. Add 150 mL of *Mobile phase*, stir for 2 h, then dilute with *Mobile phase* to volume. Centrifuge a portion of the preparation. Pipet 3.0 mL of clear supernatant into a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between ketoprofen and ketoprofen related compound A, *System suitability solution*

Tailing factor: NMT 1.5 for the ketoprofen peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ketoprofen ($C_{16}H_{14}O_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Ketoprofen RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of ketoprofen in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS**• DISSOLUTION (711)**

The *Standard solution* and *Sample solution* must be protected from light.

Medium: 0.05 M phosphate buffer, pH 7.4; 1000 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Ketoprofen RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration similar to that of the *Standard solution*.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 260 nm

Cell path length: 1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of ketoprofen ($C_{16}H_{14}O_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

A_U = absorbance from the *Sample solution*
 A_S = absorbance from the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)
 L = label claim (mg/Capsule)
 D = dilution factor of the *Sample solution*
 V = volume of *Medium*, 1000 mL

Tolerances: NLT 80% (Q) of the labeled amount of ketoprofen ($C_{16}H_{14}O_3$) is dissolved.

IMPURITIES**• ORGANIC IMPURITIES**

The *System suitability solution*, *Standard solution*, and *Sample solution* must be protected from light.

Buffer: 68.0 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5 \pm 0.05.

Mobile phase: Acetonitrile, water, and *Buffer* (43:55:2)

Diluent: Acetonitrile and water (2:3)

System suitability solution: 5 μ g/mL of USP Ketoprofen RS and 1.5 μ g/mL of USP Ketoprofen Related Compound D RS in *Diluent*

Standard solution: 2 μ g/mL of USP Ketoprofen RS, 2 μ g/mL of USP Ketoprofen Related Compound C RS, and 3 μ g/mL of USP Ketoprofen Related Compound D RS in *Diluent*

Sample solution: Nominally 1 mg/mL of ketoprofen in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 233 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: 7 times the retention time of ketoprofen

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 7.0 between ketoprofen related compound D and ketoprofen

Relative standard deviation: NMT 10% for the ketoprofen peak

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
 r_S = peak response of the corresponding related compound from the *Standard solution*
 C_S = concentration of the corresponding USP Ketoprofen Related Compound RS in the *Standard solution* (mg/mL); use the concentration of the USP Ketoprofen RS for unknown impurities
 C_U = nominal concentration of ketoprofen in the *Sample solution* (mg/mL)
Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ketoprofen related compound C ^a	0.3	0.2
Ketoprofen	1.0	—
Ketoprofen related compound D ^b	1.5	0.3
Individual unspecified impurity	—	0.2
Total impurities	—	0.5

^a 2-(3-Carboxyphenyl) propionic acid.

^b 3-Acetylbenzophenone.

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in tight containers, and store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Ketoprofen RS

(\pm)-*m*-Benzoylhydratropic acid.

$C_{16}H_{14}O_3$ 254.28

USP Ketoprofen Related Compound A RS

α -Methyl-3-(4-methylbenzoyl) benzeneacetic acid.

$C_{17}H_{16}O_3$ 268.31

USP Ketoprofen Related Compound C RS

2-(3-Carboxyphenyl) propionic acid.

$C_{10}H_{10}O_4$ 194.18

USP Ketoprofen Related Compound D RS

3-Acetylbenzophenone.

$C_{15}H_{12}O_2$ 224.25

Ketoprofen Extended-Release Capsules**DEFINITION**

Ketoprofen Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of ketoprofen ($C_{16}H_{14}O_3$).

IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- B. ULTRAVIOLET ABSORPTION (197):** The UV spectrum from the *Sample solution* in the *Analysis* for the *Dissolution* section corresponds to the spectrum from the *Standard solution*.

ASSAY**PROCEDURE**

[NOTE—Protect the *Standard solution* and *Sample solution* from light.]

Mobile phase: Acetonitrile, water, and glacial acetic acid (90:110:1)

Standard stock solution: 0.24 mg/mL of USP Ketoprofen RS in *Mobile phase*

Standard solution: 0.024 mg/mL of USP Ketoprofen RS in *Mobile phase*, from the *Standard stock solution*

System suitability solution: 0.25 mg/mL of USP Ketoprofen RS and 0.5 mg/mL of USP Ketoprofen Related Compound A RS in *Mobile phase*. Pipet 4.0 mL of this solution into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Remove completely the contents of NLT 20 Capsules, and transfer a quantity of the beads, equal to 200 mg of ketoprofen, to a 250-mL volumetric flask. Add 150 mL of *Mobile phase* and mix; bring to volume. Centrifuge, and pipet 3.0 mL of clear supernatant that contains about 2.4 mg of ketoprofen into a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.2 mL/min

Injection size: 20 μL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 3.0 between ketoprofen and ketoprofen related compound A, *System suitability solution*

Tailing factor: NMT 1.5 for the ketoprofen peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{16}H_{14}O_3$ in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ketoprofen RS in the *Standard solution* (mg/mL)

C_U = concentration of ketoprofen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS**DISSOLUTION (711)**

Medium: pH 6.8 phosphate buffer; 1000 mL

Apparatus 2: 50 rpm

Time: 1, 4, and 8 h

Detector: UV 258 nm

Standard solution: About 0.1 mg/mL of USP Ketoprofen RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 10-μm pore size, then

pass the filtrate through a suitable filter of 0.45-μm pore size.

Capsules labeled to contain 200 mg: In a test tube, dilute 5.0 mL of filtrate with 5.0 mL of *Medium*.

Capsules labeled to contain 150 mg: In a test tube, dilute 6.0 mL of filtrate with 3.0 mL of *Medium*.

Capsules labeled to contain 100 mg: No dilution is necessary.

Capsule blank: Place 10 empty, clean Capsules of the appropriate dosage into a 1000-mL volumetric flask. Add about 800 mL of *Medium* at 37°. Stir until Capsule shells are disintegrated. After equilibration to room temperature, dilute with *Medium* to volume. Transfer 100.0 mL to a 1000-mL volumetric flask, and dilute with *Medium* to volume. Pass through a suitable filter of 10-μm pore size, then pass the filtrate through a suitable filter of 0.45-μm pore size.

Capsules labeled to contain 200 mg: In a flask, dilute 25.0 mL with 25.0 mL of *Medium*.

Capsules labeled to contain 150 mg: In a flask, dilute 30.0 mL with 15.0 mL of *Medium*.

Capsules labeled to contain 100 mg: No dilution is necessary.

Analysis

Samples: *Standard solution*, *Sample solution*, and *Capsule blank*, using *Medium* as the blank

Calculate the concentration, in mg/mL, of ketoprofen in the sample withdrawn at each time point:

$$\text{Result} = (A_U - A_{CB}) \times (C_S/A_S)$$

A_U = absorbance of the *Sample solution*

A_{CB} = absorbance of the *Capsule blank*

C_S = concentration of USP Ketoprofen RS in the *Standard solution* (mg/mL)

A_S = absorbance of the *Standard solution*

Calculate the percentage of ketoprofen dissolved at each time point:

$$\text{Result} = (D + \Sigma R) \times 100/L$$

D = [amount dissolved (mg)] = volume (mL) remaining before draw × concentration (mg/mL) of sample withdrawn at the sampling time point

R = [amount removed (mg)] = volume (mL) of sample withdrawn × concentration (mg/mL) of sample withdrawn at each time point

100 = conversion factor for percentage

L = Capsule label claim (mg)

Tolerances: The percentage of the labeled amount of ketoprofen released at the times specified conforms to *Acceptance Table 2*.

Time (h)	Amount Dissolved
1	10%–25%
4	55%–80%
8	NLT 80%

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

Procedure for content uniformity: [NOTE—Protect the *Standard solution* and *Sample solution* from light.]

Mobile phase, Standard solution, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Sample solution: Transfer the contents of 10 Capsules, 1 Capsule each, to each of 10 250-mL volumetric flasks, add about 150 mL of *Mobile phase* to each flask, and stir for 2 h. Dilute with *Mobile phase* to volume, and mix. Centrifuge, and pipet a volume of clear supernatant that contains about 2.4 mg of ketoprofen into a

100-mL volumetric flask. Dilute with *Mobile phase* to volume.

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 3.0 between ketoprofen and ketoprofen related compound A, *System suitability solution*

Tailing factor: NMT 1.5 for the ketoprofen peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{16}H_{14}O_3$ in each Capsule:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Ketoprofen RS in the *Standard solution* (mg/mL)
 C_U = concentration of ketoprofen in the *Sample solution* (mg/mL)

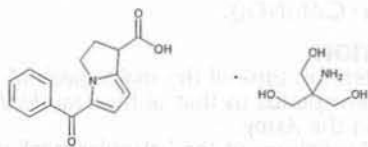
SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 3.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
 USP Ketoprofen RS
 USP Ketoprofen Related Compound A RS
 α -Methyl-3-(4-methylbenzoyl) benzeneacetic acid.

Ketorolac Tromethamine



$C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$ 376.40
 1*H*-Pyrrolizine-1-carboxylic acid, 5-benzoyl-2,3-dihydro, (\pm)-, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1);
 (\pm)-5-Benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1) [74103-07-4].

DEFINITION

Ketorolac Tromethamine contains NLT 98.5% and NMT 101.5% of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

Delete the following:

- **B. ULTRAVIOLET ABSORPTION (197U)**

Sample solution: 10 μ g/mL

Medium: Methanol

Acceptance criteria: Meets the requirements (IRA 1-May-2016)

Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. (IRA 1-May-2016)

- **C. THIN-LAYER CHROMATOGRAPHY, Tromethamine Test**

Diluent: Dichloromethane and methanol (2:1)

Standard solution: 5 mg/mL of USP Ketorolac Tromethamine RS in *Diluent*

Sample solution: 5 mg/mL of Ketorolac Tromethamine in *Diluent*

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 40 μ L

Developing solvent system: Dichloromethane, acetone, and glacial acetic acid (95:5:2)

Spray reagent: Freshly prepared alcoholic solution containing 30 mg/mL of ninhydrin

Analysis

Samples: *Standard solution* and *Sample solution*
 Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. Spray the plate with *Spray reagent*, and heat the plate at about 150° for 2–5 min.

Acceptance criteria: Yellow spots with pink to purple borders develop on the plate in the areas where the *Standard solution* and the *Sample solution* were applied.

ASSAY

- **PROCEDURE**

Protect all the solutions from light.

Buffer: 5.75 g/L of monobasic ammonium phosphate. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Tetrahydrofuran and *Buffer* (30:70)

Diluent: Tetrahydrofuran and water (30:70)

System suitability solution: In a 250-mL separator, mix 100 mL of water, 100 mL of dichloromethane, 30 mg of USP Ketorolac Tromethamine RS, and 1 mL of 1 N hydrochloric acid. Insert the stopper, shake, and allow the layers to separate. Transfer the lower dichloromethane layer to a stoppered borosilicate glass flask, and discard the upper layer. Expose the dichloromethane solution to direct sunlight for 10–15 min. Transfer 1.0 mL of the solution to a vial, evaporate in a current of air or in a stream of nitrogen to dryness, add 1.0 mL of *Diluent*, and swirl to dissolve. [NOTE—This solution may be stored under refrigeration and used as long as the chromatogram obtained as directed for *Analysis* is suitable for identifying the peaks due to the ketorolac 1-keto analog and ketorolac 1-hydroxy analog, and for the measurement of the resolution between the ketorolac 1-keto analog and ketorolac.]

Standard solution: 0.4 mg/mL of USP Ketorolac Tromethamine RS in *Diluent*

Sample solution: 0.4 mg/mL of Ketorolac Tromethamine in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 313 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for the ketorolac 1-hydroxy analog, the ketorolac 1-keto analog, and ketorolac are about 0.63, 0.89, and 1.0, respectively.]

Make adjustments, if necessary, to achieve a retention time for ketorolac of about 8–12 min.]

Suitability requirements

Resolution: NLT 1.5 between ketorolac 1-keto analog and ketorolac, *System suitability solution*

Column efficiency: NLT 5500 theoretical plates, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$) in the portion of Ketorolac Tromethamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Ketorolac Tromethamine RS in the *Standard solution* (mg/mL)

C_U = concentration of Ketorolac Tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-Jan-2018)

Change to read:

• ORGANIC IMPURITIES

Mobile phase, Diluent, *System suitability solution*, *Standard solution*, and *Sample solution*: Proceed as directed in the *Assay*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 313 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 μL

Run time: 3 times the retention time of ketorolac

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Ketorolac Tromethamine taken:

$$\text{Result} = (r_U/r_T) \times F \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity having a 0.54 relative retention time	0.54	2.2	0.5
Ketorolac 1-hydroxy analog	0.63	0.67	0.1

Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity having a 0.66 relative retention time	0.66	0.91	0.5
Ketorolac 1-keto analog	0.89	0.52	0.1
• Individual unspecified impurity	—	1.0	0.5% (IHA 1-May-2016)
Ketorolac tromethamine	1.0	1.0	—
Total impurities	—	—	1.0

SPECIFIC TESTS

• pH (791)

Sample solution: 10 mg/mL

Acceptance criteria: 5.7–6.7

• LOSS ON DRYING (731)

Analysis: Dry under vacuum at 60° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

• USP REFERENCE STANDARDS (11)

USP Ketorolac Tromethamine RS

Ketorolac Tromethamine Injection

DEFINITION

Ketorolac Tromethamine Injection is a sterile solution of Ketorolac Tromethamine. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the ketorolac peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

[NOTE—Protect all solutions from light.]

Mobile phase: Methanol, water, and glacial acetic acid (55:44:1)

Diluent: Methanol and water (1:1)

Standard solution: 0.05 mg/mL of USP Ketorolac Tromethamine RS in *Diluent*

Sample solution: Nominally equivalent to 0.05 mg/mL of ketorolac tromethamine in *Diluent* from Injection

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm. For *Identification test B*, use a diode array detector in the range of 200–600 nm.

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.2 mL/min

Injection volume: 100 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for the ketorolac peak

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$) in each mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ketorolac from the *Sample solution*

r_S = peak response of ketorolac from the *Standard solution*

C_S = concentration of USP Ketorolac Tromethamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ketorolac tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• ORGANIC IMPURITIES

[NOTE—Protect all solutions from light.]

Mobile phase, Diluent, and Chromatographic system:

Proceed as directed in the *Assay*.

Standard stock solution: 0.10 mg/mL each of USP Ketorolac Tromethamine RS, USP Ketorolac Related Compound A RS, USP Ketorolac Related Compound B RS, USP Ketorolac Related Compound C RS, and USP Ketorolac Related Compound D RS in *Diluent* prepared as follows. Transfer USP Ketorolac Tromethamine RS, USP Ketorolac Related Compound A RS, USP Ketorolac Related Compound B RS, USP Ketorolac Related Compound C RS, and USP Ketorolac Related Compound D RS to a suitable volumetric flask. Add 4% of the volume of the flask with methanol. Sonicate and dilute with *Diluent* to volume.

Standard solution: 0.2 μg/mL each of USP Ketorolac Tromethamine RS, USP Ketorolac Related Compound A RS, USP Ketorolac Related Compound B RS, USP Ketorolac Related Compound C RS, and USP Ketorolac Related Compound D RS in *Diluent* from the *Standard stock solution*

Sample solution: Prepare nominally equivalent to 0.2 mg/mL of ketorolac tromethamine in *Diluent*.

System suitability

Sample: *Standard solution*

[NOTE—See Table 1 for the relative retention times.]

Suitability requirements

Resolution: NLT 2 between ketorolac related compound C and ketorolac

Relative standard deviation: NMT 2.8% for all the peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of ketorolac related compound A, ketorolac related compound B, ketorolac related compound C, and ketorolac related compound D in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of ketorolac related compound A, ketorolac related compound B, ketorolac related compound C, or ketorolac related compound D from the *Sample solution*

r_S = peak response of ketorolac related compound A, ketorolac related compound B, ketorolac related compound C, or ketorolac related compound D from the *Standard solution*

C_S = concentration of the corresponding related compound in the *Standard solution* (mg/mL)

C_U = nominal concentration of ketorolac tromethamine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_S = peak response of ketorolac from the *Standard solution*

C_S = concentration of USP Ketorolac Tromethamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ketorolac tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ketorolac related compound A	0.4	0.20
Ketorolac related compound B	0.6	0.5
Ketorolac related compound C	0.8	0.5
Ketorolac	1.0	—
Ketorolac related compound D	2.1	0.20
Any unspecified impurity	—	0.20
Total impurities	—	1.50

SPECIFIC TESTS

• **PH (791):** 6.9–7.9

• **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 5.8 USP Endotoxin Units/mg of ketorolac tromethamine.

• **STERILITY TESTS (71):** Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*

• **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections

• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products (1)*

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, protected from light, and store at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Endotoxin RS

USP Ketorolac Tromethamine RS

USP Ketorolac Related Compound A RS

5-Benzoyl-N-[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]-2,3-dihydro-1H-pyrrolizine-1-carboxamide.

$C_{19}H_{22}N_2O_5$ 358.15

USP Ketorolac Related Compound B RS

5-Benzoyl-2,3-dihydro-1H-pyrrolizin-1-ol.

$C_{14}H_{13}NO_2$ 227.09

USP Ketorolac Related Compound C RS

5-Benzoyl-2,3-dihydro-1H-pyrrolizin-1-one.

$C_{14}H_{11}NO_2$ 225.09

USP Ketorolac Related Compound D RS
5-Benzoyl-2,3-dihydro-1H-pyrrolizine.
 $C_{14}H_{13}NO$ 211.1

Ketorolac Tromethamine Tablets

DEFINITION

Ketorolac Tromethamine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the ketorolac peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Mobile phase: Methanol, water, and glacial acetic acid (55:44:1)

Diluent: Methanol and water (1:1). [NOTE—Protect all volumetric solutions from light.]

Standard stock solution: 0.24 mg/mL of USP Ketorolac Tromethamine RS in methanol

Standard solution: 24 µg/mL of USP Ketorolac Tromethamine RS in *Diluent* from *Standard stock solution*

System suitability stock solution: 25 µg/mL each of USP Ketorolac Related Compound A RS, USP Ketorolac Related Compound B RS, USP Ketorolac Related Compound C RS, and USP Ketorolac Related Compound D RS in methanol

System suitability solution: 0.25 µg/mL each of USP Ketorolac Related Compound A RS, USP Ketorolac Related Compound B RS, USP Ketorolac Related Compound C RS, and USP Ketorolac Related Compound D RS in *Standard solution* from *System suitability stock solution*

Sample stock solution: 0.2 mg/mL of ketorolac tromethamine prepared as follows. Transfer 10 Tablets to a suitable volumetric flask. Add a quantity of water equivalent to about 10% of the volume of the flask, and sonicate until the Tablets are disintegrated. Add a quantity of methanol equivalent to 40% of the volume of the flask, and sonicate for 10 min to dissolve the ketorolac tromethamine. Cool to ambient temperature, dilute with methanol to volume, and mix. Centrifuge, or allow to settle.

Sample solution: 0.02 mg/mL of ketorolac tromethamine in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector

Assay: UV 254 nm

Identification test B: Diode array, UV 200–400 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.2 mL/min

Injection volume: 100 µL

Run time: 3.8 times the retention time of the ketorolac peak

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times are 0.8 for ketorolac related compound B and 1.0 for the ketorolac peaks.]

Suitability requirements

Resolution: NLT 1.5 each between the ketorolac and ketorolac related compound B, and ketorolac and

ketorolac related compound C peaks, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = response of the ketorolac peak from the *Sample solution*

r_S = response of the ketorolac peak from the *Standard solution*

C_S = concentration of USP Ketorolac Tromethamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ketorolac tromethamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: Water; 600 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: USP Ketorolac Tromethamine RS in *Medium*

Sample solutions: Sample per *Dissolution* (711). Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

Instrumental conditions

Mode: UV absorption spectroscopy

Analytical wavelength: 322 nm

Tolerances: NLT 75% (Q) of the labeled amount of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase, Chromatographic system, and Diluent: Proceed as directed in the *Assay*.

Standard solution: Use the *System suitability solution*, prepared as directed in the *Assay*.

Sample solution: Proceed as directed for the *Sample solution* in the *Assay*.

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 each between the ketorolac and ketorolac related compound B, and ketorolac and ketorolac related compound C peaks

Relative standard deviation: NMT 5.0% for ketorolac related compound A, ketorolac related compound B, ketorolac related compound C, and ketorolac related compound D

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each known impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each known impurity in the *Sample solution*

r_S = peak response of each known impurity in the *Standard solution*

C_S = concentration of each impurity in the *Standard solution* (mg/mL)

C_u = nominal concentration of ketorolac tromethamine in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_t) \times 100$$

r_u = response of each individual impurity peak in the *Sample solution*

r_t = sum of responses for all the peaks in the *Sample solution*

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ketorolac related compound A	0.5	0.5
Ketorolac related compound B	0.8	0.5
Ketorolac	1.0	—
Ketorolac related compound C	1.2	0.8
Ketorolac related compound D	2.6	0.5
Total unspecified impurity	—	0.5
Total impurities	—	1.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature, protected from light and excessive humidity.
- **USP REFERENCE STANDARDS (11)**
 - USP Ketorolac Tromethamine RS
 - USP Ketorolac Related Compound A RS
 - 5-Benzoyl-N-[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]-2,3-dihydro-1H-pyrrolizine-1-carboxamide.
 - $C_{19}H_{22}N_2O_5$ 358.39
 - USP Ketorolac Related Compound B RS
 - 5-Benzoyl-2,3-dihydro-1H-pyrrolizin-1-ol.
 - $C_{14}H_{13}NO_2$ 227.26
 - USP Ketorolac Related Compound C RS
 - 5-Benzoyl-2,3-dihydro-1H-pyrrolizin-1-one.
 - $C_{14}H_{11}NO_2$ 225.24
 - USP Ketorolac Related Compound D RS
 - 5-Benzoyl-2,3-dihydro-1H-pyrrolizine.
 - $C_{14}H_{13}NO$ 211.26

Krypton Kr 81m

Kr 81m

Krypton, isotope of mass 81 (metastable).

Krypton, isotope of mass 81 (metastable) [15678-91-8].

» Krypton Kr 81m is a gas suitable only for inhalation in diagnostic studies, and is obtained from a generator that contains rubidium 81 adsorbed on an immobilized suitable column support. Rubidium 81 decays with a half-life of 4.58 hours and forms its radioactive daughter ^{81m}Kr , which is eluted from the generator by passage of humidified oxygen or air through the column. Rubidium 81 is produced in an accelerator by proton bombardment of Kr 82. Other radioisotopes of rubidium are produced and are present on the generator column. These other radioisotopes do not decay to ^{81m}Kr . The column contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of Rb 81 at the date and time indicated in the labeling, and on elution yields not less than 80.0 percent of ^{81m}Kr .

Packaging and storage—The generator column is enclosed in a lead container. The unit is stored at room temperature.

Labeling—The labeling indicates the name and address of the manufacturer, the name of the generator, the quantity of ^{81}Rb at the date and time of calibration, and the statement, "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{81m}Kr is 13.1 seconds.

NOTE—Perform the following tests and Assay quickly, because of the rapid decay of the ^{81m}Kr .

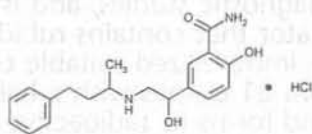
Radionuclide identification (see *Radioactivity* (821))—The gamma-ray spectrum of eluted ^{81m}Kr exhibits a monoenergetic gamma ray at a mean energy of 191 KeV.

Change to read:

Radionuclidic purity—Using a suitable counting assembly (see *Radioactivity* (821)), determine the radioactivity of each radionuclide present in a specimen of Kr 81m gas obtained from eluting the generator by use of a calibrated system as directed under *Radioactivity* (821). Not less than 99.9% of the radioactivity in the specimen eluted from the generator is present as ^{81m}Kr .

Assay for radioactivity—Using a suitable counting assembly (see *Radioactivity* (821)), determine the quantity, in MBq (mCi), of Kr 81m contained in an elution of the generator. Decay correct the result to the time of generator elution, and calculate the quantity of ^{81}Rb present in the column at the time of elution. The quantity of ^{81m}Kr eluted is not less than 80.0 percent of the labeled MBq (mCi) of ^{81}Rb present on the column at time of elution.

Labetalol Hydrochloride



$C_{19}H_{24}N_2O_3 \cdot HCl$ 364.87

Benzamide, 2-hydroxy-5-[(1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl)-, monohydrochloride. 5-[(1-Hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl)salicylamide monohydrochloride [32780-64-6].

» Labetalol Hydrochloride contains not less than 97.5 percent and not more than 101.0 percent of $C_{19}H_{24}N_2O_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards (11)—
USP Labetalol Hydrochloride RS

Identification—

A: Infrared Absorption (197M).

B: It responds to the tests for Chloride (191).

pH (791): between 4.0 and 5.0, in a solution (1 in 100).

Loss on drying (731): Dry it in a vacuum at 105° for 4 hours; it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals**, Method II (231): 0.002%. (Official 1-Jan-2018)

Chromatographic purity—

Detection reagent—Transfer 2.5 g of cadmium acetate to a 500-mL volumetric flask, add 10 mL of glacial acetic acid, dilute with alcohol to volume, and mix. Just prior to use, prepare a 0.2 in 100 solution of ninhydrin in the cadmium acetate solution for use as the *Detection reagent*.

Solvent mixture—Prepare a solution of methanol and water (4:1), and mix.

Ammonium chloride reference solution—Dissolve 60 mg of ammonium chloride in 10.0 mL of water, and mix.

Standard stock solution—Dissolve USP Labetalol Hydrochloride RS in *Solvent mixture*, and mix to obtain a solution having a known concentration of 40 mg per mL.

Standard solution 1—Quantitatively dilute a portion of the *Standard stock solution* with *Solvent mixture* to obtain a solution having a known concentration of 0.2 mg per mL.

Standard solution 2—Quantitatively dilute a portion of the *Standard solution 1* with *Solvent mixture* to obtain a solution having a known concentration of 0.1 mg per mL.

Test solution—Dissolve 200 mg of Labetalol Hydrochloride in 5.0 mL of *Solvent mixture*, and mix.

Procedure I—Apply separately 5-μL portions of the *Standard stock solution*, *Standard solution 1*, *Standard solution 2*, and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a mixture of dichloromethane, methanol, and ammonium hydroxide (15:5:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light: the R_f

value of the principal spot from the *Test solution* corresponds to that of the principal spot from the *Standard stock solution*.

Spray the plate with *Detection reagent*, heat the plate at 105° for 15 minutes, cool to room temperature, and examine the chromatogram: no individual secondary spot observed in the chromatogram of the *Test solution* is greater in size or intensity than the principal spot observed in the chromatogram of *Standard solution 1* (0.5% each). [NOTE—The spots appear as dark orange spots on a light orange to yellow background. A “negative image” spot (white) near the origin may be observed in the chromatogram of the *Test solution*. This is due to the formation of ammonium chloride during the chromatographic procedure and may be ignored.]

Procedure II—Apply separately 10-μL portions of the *Ammonium chloride reference solution*, the *Standard stock solution*, *Standard solution 1*, *Standard solution 2*, and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a mixture of ethyl acetate, isopropyl alcohol, water, and ammonium hydroxide (25:15:8:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light: no individual secondary spot (other than that due to ammonium chloride) observed in the chromatogram of the *Test solution* is greater in size or intensity than the principal spot observed in the chromatogram of *Standard solution 1* (0.5% each).

Total impurities—The sum of the intensities of all secondary spots (other than those due to ammonium chloride) observed in the chromatograms of the *Test solution* from both *Procedure I* and *Procedure II* does not exceed 1.0%.

Diastereoisomer ratio—

1-Butaneboronic acid solution—Dissolve 1-butaneboronic acid in pyridine, previously dried over a suitable molecular sieve, and mix to obtain a solution having a known concentration of 20 mg per mL.

System suitability solution—Dissolve an accurately weighed quantity of USP Labetalol Hydrochloride RS in *1-Butaneboronic acid solution*, and dilute quantitatively and stepwise with *1-Butaneboronic acid solution* to obtain a solution having a known concentration of about 1.4 mg of USP Labetalol Hydrochloride RS per mL. Allow the solution to stand at room temperature for 20 minutes before using.

Test solution—Transfer about 1 mg of Labetalol Hydrochloride to a 1-mL reaction vial, add 0.7 mL of *1-Butaneboronic acid solution*, and mix until the labetalol hydrochloride is completely dissolved. Allow the solution to stand at room temperature for 20 minutes before using.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 1.8-m glass column packed with 10% phase G3 on 100- to 120-mesh support S1AB. The column temperature is maintained at about 320°, and the injection port and the detector block temperatures are maintained at about 340°. Nitrogen is used as the carrier gas at the flow rate of about 30 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for the diastereoisomer B 1-butaneboronate derivative and 1.0 for the diastereoisomer A 1-butaneboronate derivative; the resolution, R_s , between the diastereoisomer A 1-butaneboronate derivative and diastereoisomer B 1-butaneboronate derivative peaks is not less than 1.5; and the relative standard deviation of the ratios of the peak areas of the diastereoisomers for replicate injections is not more than 2.0%.

Procedure—Inject about 2 μ L of the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the diastereoisomer A content, in percentage, taken by the formula:

$$100r_A / (r_A + r_B)$$

in which r_A is the peak area of the diastereoisomer A 1-butaneboronate derivative peak; and r_B is the peak area of the diastereoisomer B 1-butaneboronate derivative peak. The diastereoisomer A content is not less than 45.0% and not more than 55.0%.

Assay—

Mobile phase—Prepare a suitable filtered and degassed mixture of 0.1 M monobasic sodium phosphate and methanol (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Labetalol Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.4 mg per mL.

Assay preparation—Transfer about 40 mg of Labetalol Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm \times 20-cm column that contains packing L1 and is maintained at $60 \pm 1^\circ$. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 700 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 5 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$) in the portion of Labetalol Hydrochloride taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Labetalol Hydrochloride RS in the *Standard preparation*; and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Labetalol Hydrochloride Injection

» Labetalol Hydrochloride Injection is a sterile solution of Labetalol Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$).

Packaging and storage—Preserve in single-dose containers, or in multiple-dose containers not exceeding 60 mL in volume, preferably of Type I glass, at a temperature between 2° and 30° . Avoid freezing and exposure to light.

USP Reference standards (11)—

USP Endotoxin RS

USP Labetalol Hydrochloride RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test (85)—It contains not more than 1.2 USP Endotoxin Units per mg of labetalol hydrochloride.

pH (791): between 3.0 and 4.5.

Other requirements—It meets the requirements under *Injections* and *Implanted Drug Products* (1).

Assay—

Mobile phase—Prepare a suitable filtered and degassed mixture of 0.1 M monobasic sodium phosphate and methanol (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Labetalol Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL.

Resolution solution—Dissolve a quantity of methylparaben in the *Standard preparation* to obtain a solution containing about 0.08 mg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of labetalol hydrochloride, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm \times 20-cm column that contains packing L1 and is maintained at $60 \pm 1^\circ$. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 700 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for methylparaben and 1.0 for labetalol; and the resolution, R , between the methylparaben and labetalol is not less than 2.0.

Procedure—Separately inject equal volumes (about 5 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$) in each mL of the Injection taken by the formula:

$$100(C / V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Labetalol Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Labetalol Hydrochloride Compounded Oral Suspension

DEFINITION

Labetalol Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$).

Prepare Labetalol Hydrochloride Compounded Oral Suspension 40 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Labetalol Hydrochloride	4 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

Place the required number of tablets in a suitable mortar and comminute to a fine powder, or use *Labetalol Hydrochloride* powder. Add 20 mL of the *Vehicle*, and mix to form a uniform paste. Add the *Vehicle* in small portions almost to volume. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add the *Vehicle* in portions to rinse the mortar, then add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY**• PROCEDURE**

Mobile phase: Methanol and 0.1 M monobasic sodium phosphate (35:65). Filter, and degas.

Standard solution: 400 µg/mL of USP Labetalol Hydrochloride RS

Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at -70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.3 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for labetalol hydrochloride is about 7.5 min.]

Suitability requirements

Relative standard deviation: NMT 1.6% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Labetalol Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of labetalol hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• PH (791): 4.0–5.0

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.

• BEYOND-USE DATE: NMT 60 days after the date on which it was compounded when stored at controlled room temperature, or in a refrigerator

• LABELING: Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.

• USP REFERENCE STANDARDS (11)

USP Labetalol Hydrochloride RS

Labetalol Hydrochloride Tablets

» Labetalol Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers, at a temperature between 2° and 30°.

USP Reference standards (11)—

USP Labetalol Hydrochloride RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{19}H_{24}N_2O_3 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 302 nm of filtered portions of the solution under test, suitably diluted with water, if necessary, in comparison with a *Standard solution* having a known concentration of USP Labetalol Hydrochloride RS in the same *Medium*.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{19}H_{24}N_2O_3 \cdot HCl$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay* under *Labetalol Hydrochloride*.

Assay preparation—Transfer an accurately counted number of Tablets, equivalent to about 2000 mg of labetalol hydrochloride, to a 500-mL volumetric flask, add 200 mL of water, and shake by mechanical means for 60 minutes. Dilute with water to volume, and mix. Filter the solution through a filter of 0.5 µm or finer porosity, discarding the first few mL of the filtrate. Transfer 10.0 mL of the filtrate to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$) in each Tablet taken by the formula:

$$5000(C/N)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Labetalol Hydrochloride RS in the *Standard preparation*; N is the number of Tablets taken; and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Lactase

DEFINITION

Lactase (β -D-galactoside galactohydrolase) is a hydrolytic enzyme derived from the mold *Aspergillus oryzae*. It contains NLT 30,000 USP Lactase Units/g.

[NOTE—One USP Lactase Unit is the lactase activity contained in the amount of enzyme that hydrolyzes one microequivalent of galactosidic linkage per min at a pH of 4.5 and at 37°, as directed in the Assay for Lactase Activity.]

ASSAY

• LACTASE ACTIVITY

Solution A: Dilute 57.5 mL of glacial acetic acid with sufficient water to make a 500-mL solution. Transfer 50 mL of the glacial acetic acid solution into a 1000-mL volumetric flask, add 11.3 mL of 4 N sodium hydroxide, and dilute with water to volume. If necessary, adjust with glacial acetic acid solution or 4 N sodium hydroxide to a pH of 4.50 ± 0.05 .

Substrate solution: On the day of use, weigh 370.0 mg of *o*-nitrophenyl- β -D-galactopyranoside, and place in a 100-mL volumetric flask. Add about 50 mL of *Solution A*, swirl to dissolve, then dilute with *Solution A* to volume.

Standard solution: Transfer about 0.4 g of USP Lactase RS, accurately weighed, to a 1000-mL volumetric flask. Add about 600 mL of water, allow to stand for 15 min, swirl gently, and dilute with water to volume. Pipet 3.0 mL of this solution into a 200-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer an accurately weighed quantity of about 0.4 g of Lactase to a 1000-mL volumetric flask. Add about 600 mL of water, allow to stand for 15 min, swirl gently, and dilute with water to volume. Pipet 3.0 mL of this solution into a 200-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: Vis

Analytical wavelength: 420 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Pipet 2.0 mL of *Substrate solution* into three separate test tubes labeled S, U, and B. Transfer the tubes to a thermostated water bath maintained at $37.0 \pm 0.1^\circ$, and incubate for 10 min. Following the incubation, rapidly add 0.5 mL of the *Standard solution* to tube S, 0.5 mL of the *Sample solution* to tube U, and 0.5 mL of water to tube B (the reagent blank). Mix each tube on a vortex mixer for 1 s, and immediately return the tubes to the water bath, which has been maintained at $37.0 \pm 0.1^\circ$. After 15 min of incubation, rapidly add 2.5 mL of a 10% sodium carbonate solution to each test tube to stop the enzyme reaction. Add 20.0 mL of water to each test tube, and mix. Concomitantly determine the absorbances of the three solutions.

Calculate the number of USP Lactase Units in the portion of Lactase taken:

$$\text{Result} = [(A_U - A_B)/(A_S - A_B)] \times P \times (W_S/W_U)$$

A_U = absorbance of the *Sample solution* (tube U)

A_B = absorbance of the reagent blank (tube B)

A_S = absorbance of the *Standard solution* (tube S)

P = potency of USP Lactase RS (USP Units/g)

W_S = weight of USP Lactase RS in the *Standard solution* (g)

W_U = weight of Lactase in the *Sample solution* (g)

Acceptance criteria: NLT 30,000 USP Lactase Units/g

IMPURITIES

- **ARSENIC** (211): NMT 3 $\mu\text{g/g}$
- **LEAD** (251): NMT 5 $\mu\text{g/g}$

Delete the following:

- **HEAVY METALS** (231): NMT 30 $\mu\text{g/g}$ (Official 1-Jan-2018)

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

- **LOSS ON DRYING** (731)

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 6.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers at room temperature.
- **LABELING:** Label it to indicate lactase activity in USP Units.
- **USP REFERENCE STANDARDS** (11)
USP Lactase RS

Lactic Acid

Propanoic acid, 2-hydroxy-;
Lactic acid [50-21-5].

DEFINITION

Lactic Acid is a mixture of lactic acid ($\text{C}_3\text{H}_6\text{O}_3$) and lactic acid lactate ($\text{C}_6\text{H}_{10}\text{O}_5$), equivalent to a total of NLT 88.0% and NMT 92.0%, by weight, of lactic acid ($\text{C}_3\text{H}_6\text{O}_3$). It is obtained by the lactic fermentation of sugars or is prepared synthetically. Lactic Acid obtained by fermentation of sugars is levorotatory, whereas that prepared synthetically is racemic.

[NOTE—Lactic Acid prepared by fermentation becomes dextrorotatory on dilution, which hydrolyzes L-(–)-lactic acid lactate to L-(+)-lactic acid.]

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Lactate** (191): Meets the requirements

ASSAY

• PROCEDURE

Sample: 2.5 mL, accurately weighed

Titrimetric system

(See *Titrimetry* (541).)

Mode: Residual titration

Titrant: 1 N sodium hydroxide VS

Back-titrant: 1 N sulfuric acid VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a tared 250-mL flask, add 50.0 mL of *Titrant*, and boil the mixture for 20 min. Add phenolphthalein TS, and titrate the excess alkali in the hot solution with *Back-titrant*. Perform a blank determination. Each mL of *Titrant* is equivalent to 90.08 mg of lactic acid ($\text{C}_3\text{H}_6\text{O}_3$).

Acceptance criteria: 88.0%–92.0% (w/w)

IMPURITIES

• CHLORIDE

Sample solution: 1 in 100

Analysis: To 10 mL of the *Sample solution* acidified with nitric acid add a few drops of silver nitrate TS.

Acceptance criteria: No opalescence is produced immediately.

• **SULFATE**

Sample solution: 1 in 100

Analysis: To 10 mL of the *Sample solution* add 2 drops of hydrochloric acid and 1 mL of barium chloride TS.

Acceptance criteria: No turbidity is produced.

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 10 µg/g • (Official 1-

Jan-2018)

- **RESIDUE ON IGNITION (281)**

Sample: 5 mL, accurately weighed

Acceptance criteria: NMT 3 mg (0.05%)

- **LIMIT OF CITRIC, OXALIC, PHOSPHORIC, OR TARTARIC ACID**

Sample solution: 1 in 10

Analysis: To 10 mL of the *Sample solution* add 40 mL of calcium hydroxide TS, and boil for 2 min.

Acceptance criteria: No turbidity is produced.

SPECIFIC TESTS

- **READILY CARBONIZABLE SUBSTANCES**

Sample: 5 mL

Analysis: Rinse a test tube with sulfuric acid, and allow to drain for 10 min. Add 5 mL of sulfuric acid to the test tube, carefully overlay it with the *Sample*, and maintain the tube at 15°.

Acceptance criteria: No dark color develops at the interface of the two acids within 15 min.

- **OPTICAL ROTATION, Angular Rotation (781A):** −0.05° to +0.05° for racemic Lactic Acid

- **SUGARS**

Sample: 5 drops

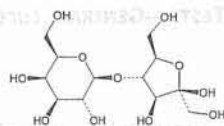
Analysis: To 10 mL of hot alkaline cupric tartrate TS add the *Sample*.

Acceptance criteria: No red precipitate is formed.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
• **LABELING:** Label it to indicate whether it is levorotatory or racemic.

Lactulose Concentrate



$C_{12}H_{22}O_{11}$ 342.30
D-Fructose, 4-O-β-D-galactopyranosyl-;
4-O-β-D-Galactopyranosyl-D-fructofuranose [4618-18-2].

DEFINITION

Lactulose Concentrate is a solution of sugars prepared from Lactose. It consists principally of lactulose together with minor quantities of lactose and galactose, and traces of other related sugars and water. It contains NLT 95.0% and NMT 105.0% of the labeled amount of lactulose ($C_{12}H_{22}O_{11}$). It contains no added substances.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

- **B.** Sample solution: Dilute a portion of Concentrate with water (1 in 20).

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A red precipitate of cuprous oxide is formed.

ASSAY

• **PROCEDURE**

Buffer: 1.15 g/L of monobasic sodium phosphate in water

Mobile phase: Acetonitrile and Buffer (82:18). Ensure that the concentration of acetonitrile in the *Mobile phase* is between 78% and 85% to obtain appropriate retention times.

Standard solution: 40 mg/mL of USP Lactulose RS, 4.8 mg/mL of USP Anhydrous Lactose RS, and 3.2 mg/mL of USP Epilactose RS in a mixture of acetonitrile and water (1:1)

Sample solution: Nominally equivalent to 40 mg/mL of lactulose prepared as follows. Transfer a quantity of Concentrate containing 2.0 g of lactulose to a 50-mL volumetric flask, and dissolve in 20 mL of water. Add 25.0 mL of acetonitrile, allow the solution to reach ambient temperature, and dilute with water to volume.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: Refractive index

Column: 4.6-mm × 15-cm; 3-µm packing L8

Temperatures

Column: 40 ± 1°

Detector: 40 ± 1°

Flow rate: 1.3 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times are given in Table 1.]

Suitability requirements

Resolution: NLT 1.5 between lactulose and lactose;
NLT 0.9 between lactulose and epilactose

Relative standard deviation: NMT 2.0% for the main peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lactulose ($C_{12}H_{22}O_{11}$) in the portion of Concentrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lactulose RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lactulose in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

- **ORGANIC IMPURITIES**

Buffer, Mobile phase, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay. To evaluate the *Suitability requirements*, use the *Standard solution* prepared as directed in the Assay.

Standard solution: 6.4 mg/mL of USP Galactose RS, 4.8 mg/mL of USP Anhydrous Lactose RS, 3.2 mg/mL of USP Epilactose RS, 1.2 mg/mL of USP Tagatose RS, and 0.4 mg/mL of USP Fructose RS in a mixture of acetonitrile and water (1:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of galactose, lactose, epilactose, tagatose, and fructose, if found, in the portion of Concentrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of the relevant related compound from the *Sample solution*
 r_S = peak response of the relevant related compound from the *Standard solution*
 C_S = concentration of the relevant USP Reference Standard in the *Standard solution* (mg/mL)
 C_U = nominal concentration of lactulose in the *Sample solution* (mg/mL)
 Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tagatose	0.30	4
Fructose	0.34	1
Galactose	0.47	16
Epilactose	0.90	8
Lactulose	1.0	—
Lactose	1.17	12

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, preferably at a temperature between 2° and 30°. Avoid subfreezing temperatures.
- **LABELING:** The label states that this article is not intended for direct administration to humans or animals.
- **USP REFERENCE STANDARDS (11)**
 - USP Epilactose RS
 - USP Fructose RS
 - USP Galactose RS
 - USP Anhydrous Lactose RS
 - USP Lactulose RS
 - USP Tagatose RS

Lactulose Solution**DEFINITION**

Lactulose Solution is a solution in water prepared from Lactulose Concentrate. It contains NLT 90.0% and NMT 110.0% of the labeled amount of lactulose ($C_{12}H_{22}O_{11}$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.**
 - Sample solution:** Dilute a portion of *Solution* with water (1 in 20).
 - Analysis:** Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.
 - Acceptance criteria:** A red precipitate of cuprous oxide is formed.

ASSAY**• PROCEDURE**

Buffer: 1.15 g/L of monobasic sodium phosphate in water
Mobile phase: Acetonitrile and *Buffer* (82:18). Ensure that the concentration of acetonitrile in the *Mobile phase* is between 78% and 85% to obtain appropriate retention times.
Standard solution: 40 mg/mL of USP Lactulose RS, 4.8 mg/mL of USP Anhydrous Lactose RS, and 3.2 mg/mL of USP Epilactose RS in a mixture of acetonitrile and water (1:1)
Sample solution: Nominally equivalent to 40 mg/mL of lactulose prepared as follows. Transfer a quantity of So-

lution containing 2.0 g of lactulose to a 50-mL volumetric flask, and dissolve in 20 mL of water. Add 25.0 mL of acetonitrile, allow the solution to reach ambient temperature, and dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 4.6-mm × 15-cm; 3-μm packing L8

Temperatures

Column: 40 ± 1°

Detector: 40 ± 1°

Flow rate: 1.3 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times are given in Table 1.]

Suitability requirements

Resolution: NLT 1.5 between lactulose and lactose;

NLT 0.9 between lactulose and epilactose

Relative standard deviation: NMT 2.0% for the main peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lactulose ($C_{12}H_{22}O_{11}$) in the portion of *Solution* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Lactulose RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of lactulose in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS**• UNIFORMITY OF DOSAGE UNITS (905)**

Oral *Solution* packaged in single-unit containers

Acceptance criteria: Meets the requirements

IMPURITIES**• ORGANIC IMPURITIES**

Buffer, Mobile phase, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*. To evaluate the *Suitability requirements*, use the *Standard solution* prepared as directed in the *Assay*.

Standard solution: 6.4 mg/mL of USP Galactose RS, 4.8 mg/mL of USP Anhydrous Lactose RS, 3.2 mg/mL of USP Epilactose RS, 1.2 mg/mL of USP Tagatose RS, and 0.4 mg/mL of USP Fructose RS in a mixture of acetonitrile and water (1:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of galactose, lactose, epilactose, tagatose, and fructose, if found, in the portion of *Solution* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of the relevant related compound from the *Sample solution*
 r_S = peak response of the relevant related compound from the *Standard solution*
 C_S = concentration of the relevant USP Reference Standard in the *Standard solution* (mg/mL)
 C_U = nominal concentration of lactulose in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

Table 1

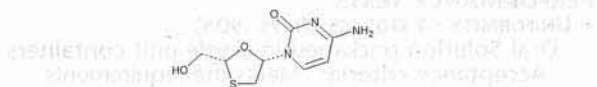
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tagatose	0.30	4
Fructose	0.34	1
Galactose	0.47	16
Epilactose	0.90	8
Lactulose	1.0	—
Lactose	1.17	12

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total bacterial count is NMT 10^2 cfu/g of lactulose, and the tests for *Salmonella* species and *Escherichia coli* are negative.
- **PH (791):** 2.5–6.5, after 15 min of contact with the electrodes

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, preferably at a temperature between 2° and 30°. Avoid subfreezing temperatures.
- **USP REFERENCE STANDARDS (11)**
 - USP Epilactose RS
 - USP Fructose RS
 - USP Galactose RS
 - USP Anhydrous Lactose RS
 - USP Lactulose RS
 - USP Tagatose RS

Lamivudine

$C_8H_{11}N_3O_3S$	229.26
$C_8H_{11}N_3O_3S \cdot 0.2 CH_3OH$	235.66
$C_8H_{11}N_3O_3S \cdot 0.2 H_2O$	232.86
2(1 <i>H</i>)-Pyrimidinone, 4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-, (2 <i>R</i> - <i>cis</i>)-;	
(-)-1-[(2 <i>R</i> ,5 <i>S</i>)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine [134678-17-4].	

DEFINITION

Lamivudine contains NLT 98.0% and NMT 102.0% of lamivudine ($C_8H_{11}N_3O_3S$), calculated on the anhydrous and solvent-free basis. If labeled as a methanol solvate, it contains NLT 98.0% and NMT 102.0% of lamivudine ($C_8H_{11}N_3O_3S$), calculated on the anhydrous, methanol-free, and solvent-free basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Limit of Lamivudine Enantiomer*.

ASSAY**• PROCEDURE**

Buffer: Transfer about 1.9 g of ammonium acetate to a 1000-mL volumetric flask, dissolve in about 900 mL of water, adjust with acetic acid to a pH of 3.8 ± 0.2 , and dilute with water to volume.

Mobile phase: Methanol and Buffer (5:95)

System suitability solution: 0.25 mg/mL of USP Lamivudine Resolution Mixture B RS in *Mobile phase*

Standard solution: 0.25 mg/mL of USP Lamivudine RS in *Mobile phase*

Sample solution: 0.25 mg/mL of Lamivudine in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 277 nm

Column: 4.6-mm \times 25-cm; packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for lamivudine diastereomer and lamivudine are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between lamivudine and lamivudine diastereomer, *System suitability solution*

Relative standard deviation: NMT 2.0% for lamivudine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lamivudine ($C_8H_{11}N_3O_3S$) in the portion of Lamivudine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)

C_U = concentration of Lamivudine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

If labeled as a methanol solvate: 98.0%–102.0% on the anhydrous, methanol-free, and solvent-free basis

OTHER COMPONENTS

- **CONTENT OF METHANOL** (if labeled as lamivudine methanol solvate)

Diluent: *N,N*-Dimethylformamide and *t*-butanol (500:1)

Standard solution: 0.625 mg/mL of methanol in diluent. Transfer 2.0 mL of this solution into a headspace vial, and immediately seal the vial.

Sample solution: Transfer 50 mg of Lamivudine to a headspace vial, add 2.0 mL of *Diluent*, and immediately seal the vial.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC headspace

Detector: Flame ionization

Column: 0.53-mm \times 75-m, coated with a 3- μ m film of phase G43

Temperatures

Injector: 180°

Detector: 250°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	13
40	40	240	12

Injection volume: 1.0 mL

Injection type: Split ratio, 15:1

Carrier gas: Helium

Flow rate: 6 mL/min

Headspace samplers

Oven: 95°

Loop: 175°

Transfer line: 175°

Equilibrium time: 10 min

System suitability

Sample: Standard solution

[NOTE—The relative retention times for methanol and *t*-butanol are 1.0 and 1.9, respectively.]

Suitability requirements

Tailing factor: NMT 2.0 for methanol

Column efficiency: NLT 25,000 for methanol

Relative standard deviation: NMT 5.0% for methanol

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of methanol in the portion of lamivudine methanol solvate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of methanol to *t*-butanol from the Sample solution

R_S = peak response ratio of methanol to *t*-butanol from the Standard solution

C_S = concentration of methanol in the Standard solution (mg/mL)

C_U = concentration of Lamivudine (as methanol solvate) in the Sample solution (mg/mL)

Acceptance criteria: 2.0%–3.0%

IMPURITIES

• LIMIT OF LAMIVUDINE ENANTIOMER

Buffer: 7.7 g/L of ammonium acetate in water

Mobile phase: Methanol and Buffer (5:95)

System suitability solution: 0.25 mg/mL of USP

Lamivudine Resolution Mixture A RS in water

Sample solution: 0.25 mg/mL of Lamivudine in water

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; packing L45

Column temperature: 15°–30° (constant temperature)

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: System suitability solution

[NOTE—The relative retention times for lamivudine and the lamivudine enantiomer are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 1.5 between lamivudine and the lamivudine enantiomer

Analysis

Sample: Sample solution

Calculate the percentage of the lamivudine enantiomer in the portion of Lamivudine taken:

$$\text{Result} = [r_U/(r_U + r_S)] \times 100$$

r_U = peak response of the lamivudine enantiomer

r_S = peak response of lamivudine

Acceptance criteria: NMT 0.3%

• OTHER RELATED COMPOUNDS

Buffer, Mobile phase, System suitability solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Salicylic acid standard solution: 0.625 µg/mL of USP

Salicylic Acid RS in Mobile phase

Sample solution: 0.25 mg/mL of Lamivudine in Mobile phase

Analysis

Samples: Salicylic acid standard solution and Sample solution

Calculate the percentage of salicylic acid in the portion of Lamivudine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid from the Sample solution

r_S = peak response of USP Salicylic Acid RS from the Salicylic acid standard solution

C_S = concentration of salicylic acid in the Salicylic acid standard solution (mg/mL)

C_U = concentration of Lamivudine in the Sample solution (mg/mL)

Calculate the percentage of other individual impurities in the portion of Lamivudine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity other than salicylic acid from the Sample solution

r_T = sum of the responses of all the peaks

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lamivudine-carboxylic acid ^a	0.4	0.3
Lamivudine-trans (lamivudine diastereomer) ^b	0.9	0.2
Lamivudine	1.0	—
Salicylic acid	2.7	0.1
Any other individual impurity	—	0.1
Total impurities	—	0.6

^a (2*RS*,5*SR*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.

^b 1-[(2*RS*,5*SR*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

• RESIDUAL SOLVENTS

Internal standard solution: Dilute 1 mL of 2-pentanol with dimethyl sulfoxide and water (1:1) to 100.0 mL.

Standard solution: Transfer 10 mL of the Internal standard solution to a 100-mL volumetric flask. Add 100 µL each of the following: dehydrated alcohol, isopropyl acetate, methanol, and triethylamine. Dilute with dimethyl sulfoxide and water (1:1) to volume.

Sample solution: Transfer 5 g of Lamivudine to a 100-mL volumetric flask, add 10 mL of the Internal standard solution, and dilute with dimethyl sulfoxide and water (1:1) to volume.

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 50-m, coated with a 5-µm film of phase G1

Temperatures

Injector: 150°

Detector: 250°

Column: See Table 3.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	—	70	3
70	30	200	6.5

Injection volume: 0.5 µL

Injection type: Split flow rate, 320 mL/min

Carrier gas: Hydrogen (at pressure 5 psig)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each residual solvent in the portion of Lamivudine taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the respective analyte to the internal standard from the *Sample solution*

R_S = peak response ratio of the respective analyte to the internal standard from the *Standard solution*

C_S = concentration of the respective analyte in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: See Table 4.

Table 4

Name	Acceptance Criteria, NMT (%)
Alcohol	0.2
Isopropyl acetate	0.2
Methanol	0.1
Triethylamine	0.1
Total residual solvents	0.3

SPECIFIC TESTS

- **WATER DETERMINATION**, Method 1c (921): NMT 2.0%

- **LIGHT ABSORPTION**

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: Vis

Sample solution: 50 mg/mL in water

Analytical wavelength: 440 nm

Cell: 4 cm

Acceptance criteria: Absorptivity NMT 0.0015

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at room temperature.
- **LABELING:** Where it is a methanol solvate form, the label so indicates.
- **USP REFERENCE STANDARDS (11)**
 - USP Lamivudine RS
 - USP Lamivudine Resolution Mixture A RS
 - USP Lamivudine Resolution Mixture B RS
 - USP Salicylic Acid RS

Lamivudine Oral Solution

DEFINITION

Lamivudine Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$). It may contain a suitable preservative.

IDENTIFICATION

- **A.** The retention time of the lamivudine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Solution A: 2.0 g/L of sodium heptanesulfonate in water. Add 1.0 mL of hydrochloric acid and 1.0 mL of triethylamine per L of the solution.

Solution B: Acetonitrile and *Solution A* (50:50)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	60	40
30	10	90
33	10	90
34	100	0
45	100	0

Diluent: Acetonitrile and water (10:90)

System suitability solution: Dissolve the contents of 1 vial of USP Lamivudine Resolution Mixture C RS in 2.5 mL of *Diluent*.

Standard solution: 0.2 mg/mL of USP Lamivudine RS in *Diluent*

Sample solution: Nominally 0.2 mg/mL of lamivudine in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 277 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between lamivudine-S-sulfoxide and lamivudine-R-sulfoxide, *System suitability solution*

Tailing factor: NMT 2.0 for the lamivudine peak, *System suitability solution*

Relative standard deviation: NMT 2% for the lamivudine peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lamivudine from the *Sample solution*

r_S = peak response of lamivudine from the *Standard solution*

C_S = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamivudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DELIVERABLE VOLUME** (698): Meets the requirements

IMPURITIES

• ORGANIC IMPURITIES

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Sample: *Sample solution*

Calculate the percentage of any individual impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times 100$$

r_U = peak response of each individual impurity

r_S = sum of the responses of all of the peaks excluding peaks due to added preservative(s) or excipients

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lamivudine-uracil derivative ^a	0.34	1.2
Cytosine ^b	0.52	0.3
Lamivudine-S-sulfoxide ^c	0.61	0.3
Lamivudine-R-sulfoxide ^d	0.63	0.6
Lamivudine carboxylic acid ^{e,f}	0.89	—
Lamivudine <i>trans</i> - ^g	0.94	—
Lamivudine	1.0	—
Salicylic acid ^f	1.38	—
Any other identified impurity	—	0.3
Any individual unidentified impurity	—	0.2
Total impurities	—	2.0

^a 1-[(2R,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil.

^b 4-Aminopyrimidin-2(1H)-one.

^c 1-[(2R,3S,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine S-oxide.

^d 1-[(2R,3R,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine S-oxide.

^e (2R,5S)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.

^f This impurity is controlled in the drug substance and is not to be included in the total impurities. Disregard any peak less than 0.01%.

^g 1-[(2S,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

SPECIFIC TESTS

- **pH** (791): 5.7–6.3

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed 10^2 cfu/mL. The total molds and yeasts count does not exceed 10^2 cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant containers at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
 - USP Lamivudine RS
 - USP Lamivudine Resolution Mixture C RS

[NOTE—This reference standard contains lamivudine and several related impurities.]

Lamivudine Tablets

DEFINITION

Lamivudine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197M)

Sample: Crush 1 Tablet and transfer it to a suitable container. Add 5 mL of methanol and shake for 15 min. Pass through a suitable filter, collecting about 2 mL of the filtrate. Evaporate the filtrate to dryness under a gentle stream of nitrogen, and use the residue.

Standard: Dissolve a suitable amount of USP Lamivudine RS in a small amount of methanol, shaking until completely dissolved. Evaporate to dryness under a gentle stream of nitrogen, and use the residue.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Buffer: 1.9 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 3.8.

Mobile phase: Methanol and Buffer (50:950)

System suitability solution: 0.2 mg/mL of USP

Lamivudine Resolution Mixture B RS in *Mobile phase*

Standard solution: 0.2 mg/mL of USP Lamivudine RS in *Mobile phase*

Sample stock solution: Nominally about 3–4 mg/mL of lamivudine in water prepared as follows. Transfer the required number of Tablets, based on the labeled amount, to a suitable volumetric flask, and soak or shake for at least 15 min in water to disperse the sample. Dilute with water to volume, mix, and pass through a suitable filter or centrifuge.

Sample solution: Nominally 0.2 mg/mL of lamivudine in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 277 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: $30 \pm 5^\circ$

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for lamivudine diastereomer and lamivudine are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between lamivudine and lamivudine diastereomer, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamivudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Procedure for products labeled as Lamivudine Tablets 100-mg or 150-mg

Medium: Water, degassed; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: (L/900) mg/mL of USP

Lamivudine RS in Medium, where L is the Tablet label claim in mg

Sample solution: Pass a portion of the solution under test through a suitable filter to obtain a concentration similar to that of the Standard solution.

Instrumental conditions

Mode: UV

Analytical wavelength: 270 nm

Cell: 1 mm

Blank: Medium

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the Sample solution

A_S = absorbance of the Standard solution

C_S = concentration of USP Lamivudine RS in the Standard solution (mg/mL)

V = volume of Medium, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) is dissolved.

Procedure for products labeled as Lamivudine Tablets 300-mg

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 75 rpm

Time: 15 min

Standard solution: (L/900) mg/mL of USP

Lamivudine RS in Medium, where L is the Tablet label claim in mg

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

Mode: UV

Analytical wavelength: 280 nm

Cell: 0.5 mm

Blank: Medium

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the Sample solution

A_S = absorbance of the Standard solution

C_S = concentration of USP Lamivudine RS in the Standard solution (mg/mL)

V = volume of Medium, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 15 min

Buffer: 1.93 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 3.8.

Mobile phase: Methanol and Buffer (40:60)

Standard solution: (L/900) mg/mL of USP Lamivudine RS in Medium, where L is the Tablet label claim in mg

Sample solution: Pass a portion of the solution under test through a suitable filter to obtain a concentration similar to that of the Standard solution.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: 285 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 5 μL

System suitability

Sample: Standard solution

Suitability requirements

Relative standard deviation: NMT 2.0%

Tailing factor: NMT 1.5

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Lamivudine RS in the Standard solution (mg/mL)

V = volume of Medium, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Sample: Sample solution

Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity

r_T = sum of all the peak responses

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cytosine ^a	0.32	0.2
Lamivudine-carboxylic acid ^b	0.39	— ^c
Lamivudine-S-sulfoxide ^d	0.43	0.2
Lamivudine-R-sulfoxide ^e	0.45	0.2
Lamivudine diastereomer (Lamivudine-trans) ^f	0.92	— ^c
Lamivudine	1.0	—

^a 4-Aminopyrimidin-2(1H)-one.

^b (2R,5R)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.

^c Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities for the drug product.

^d 1-[(2R,3S,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine S-oxide.

^e 1-[(2R,3R,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine S-oxide.

^f 1-[(2R,5R)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid	2.2	— ^c
Any other individual impurity	—	0.2
Total impurities	—	0.6

^a 4-Aminopyrimidin-2(1*H*)-one.^b (2*RS*,5*SR*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.^c Process impurity included in the table for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities for the drug product.^d 1-[(2*R*,3*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine 5-oxide.^e 1-[(2*R*,3*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine 5-oxide.^f 1-[(2*RS*,5*RS*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
USP Lamivudine RS
USP Lamivudine Resolution Mixture B RS
[NOTE—The resolution mixture contains lamivudine and lamivudine diastereomer. Other impurities may also be present.]

Lamivudine and Zidovudine Tablets**DEFINITION**

Lamivudine and Zidovudine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) and zidovudine ($C_{10}H_{13}N_5O_4$).

IDENTIFICATION

- **A.** The retention times of the lamivudine and zidovudine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: 25 mM of ammonium acetate. Adjust with glacial acetic acid to a pH of 4.0.

Solution B: Methanol

Solution C: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	95	5	0
15.0	95	5	0
30.0	70	30	0
38.0	70	30	0
38.1	0	0	100
45.0	0	0	100
45.1	95	5	0
60.0	95	5	0

Diluent: *Solution A* and *Solution B* (19:1)

System suitability solution: 0.17 mg/mL of USP Lamivudine Resolution Mixture B RS in *Diluent*

Standard solution: 0.15 mg/mL of USP Lamivudine RS and 0.30 mg/mL of USP Zidovudine RS in *Diluent*

Sample stock solution: Transfer a counted number of Tablets, equivalent to 1500 mg of zidovudine and 750 mg of lamivudine, into a 500-mL volumetric flask. Add 250 mL of water, and disintegrate completely by shaking for a minimum of 15 min. Dilute with water to volume, and mix.

Sample solution: Pass a portion of the *Sample stock solution* through a filter of 0.45- μ m pore size, discarding the first 2–3 mL. Further dilute the filtrate to obtain 0.15 mg/mL of lamivudine and 0.30 mg/mL of zidovudine in *Diluent*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 3-mm \times 25-cm; packing L1

Flow rate: 0.5 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for lamivudine diastereomer and lamivudine are 0.50 and 0.52, respectively.]

Suitability requirements

Resolution: NLT 1.5 between lamivudine diastereomer and lamivudine, *System suitability solution*

Relative standard deviation: NMT 2.0% for zidovudine and lamivudine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lamivudine ($C_8H_{11}N_3O_3S$) and zidovudine ($C_{10}H_{13}N_5O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of zidovudine or lamivudine from the *Sample solution*

r_S = peak response of zidovudine or lamivudine from the *Standard solution*

C_S = concentration of USP Zidovudine RS or USP Lamivudine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of zidovudine or lamivudine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)****Test 1**

Medium: 0.1 N hydrochloric acid; 900 mL, deaerated

Apparatus 2: 75 rpm

Time: 15 min

Lamivudine response factor solutions: 0.167 mg/mL of USP Lamivudine RS in *Medium*. [NOTE—Prepare in duplicate.]

Zidovudine response factor solutions: 0.333 mg/mL of USP Zidovudine RS in *Medium*. [NOTE—Prepare in duplicate.]

Sample solution: Pass a portion of the solution under test through a suitable filter (PTFE, PVDF, or equivalent) of 0.45- μ m pore size.

Detector: UV 240–300 nm

Blank: *Medium*

Cell length: 0.02-cm flowcell

Analysis: The calculations of the percentages dissolved are done using a multicomponent analysis software.

Tolerances: NLT 85% (Q) of the labeled amount of zidovudine and lamivudine is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 75 rpm

Time: 30 min

Buffer solution: 7.7 g/L of ammonium acetate in water

Mobile phase: Acetonitrile and Buffer solution (1:9)

Standard stock solution: 1.4 mg/mL of USP

Lamivudine RS and 2.8 mg/mL of USP Zidovudine RS in Medium. A small amount of methanol, NMT 20% of the final volume, can be used to dissolve both compounds.

Standard solution: 0.168 mg/mL of lamivudine and 0.336 mg/mL of zidovudine in Medium from the Standard stock solution

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 15-cm; packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Sample: Standard solution

Suitability requirements

Column efficiency: NLT 1500 theoretical plates for lamivudine and NLT 3000 theoretical plates for zidovudine

Tailing factor: NMT 2.0 for lamivudine and zidovudine

Relative standard deviation: NMT 2.0% for zidovudine and lamivudine

Calculate the percentages of lamivudine ($C_8H_{11}N_3O_3S$) and zidovudine ($C_{10}H_{13}N_5O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of lamivudine or zidovudine from the Sample solution

r_S = peak response of lamivudine or zidovudine from the Standard solution

C_S = concentration of lamivudine or zidovudine in the Standard solution (mg/mL)

L = label claim for lamivudine or zidovudine (mg/Tablet)

V = volume of Medium, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of zidovudine and lamivudine is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for zidovudine and lamivudine

IMPURITIES

• ORGANIC IMPURITIES

Solution A, Solution B, Solution C, Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Sample: Sample solution

Calculate the percentage of each lamivudine related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each individual impurity from the Sample solution

r_T = sum of the peak responses of lamivudine and all lamivudine related impurities from the Sample solution

Calculate the percentage of each zidovudine related impurity and unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the Sample solution

r_T = sum of the peak responses of zidovudine, all zidovudine related impurities, and unspecified impurities from the Sample solution

F = relative response factor (see Table 2)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lamivudine-(cytosine) ^a	0.11	1.0	— ^b
Lamivudine-(uracil) ^c	0.14	1.0	— ^b
Lamivudine-(carboxylic acid) ^d	0.17	1.0	0.3
Lamivudine-(S-sulfoxide) ^e	0.20	1.0	— ^b
Lamivudine-(R-sulfoxide) ^f	0.22	1.0	— ^b
Zidovudine related compound C ^g	0.27	1.7	1.5
Lamivudine diastereomer ^h	0.50	1.0	0.2
Lamivudine	0.52	—	—
Zidovudine-(thymidine) ⁱ	0.60	1.0	— ^b
Lamivudine-(uracil derivative) ^j	0.70	1.0	— ^b
Lamivudine-(salicylic acid) ^k	0.80	1.0	— ^b
Zidovudine	1.00	—	—
Zidovudine related compound B ^l	1.10	1.0	— ^b
Any individual unspecified impurity	—	1.0	0.1
Total lamivudine related impurities (the limit includes all lamivudine related impurities)	—	—	0.6
Total zidovudine related impurities (the limit includes individual unspecified impurities)	—	—	2.0

^a 4-Aminopyrimidin-2(1H)-one.

^b The individual impurity limit is not included because these are process/other impurities monitored individually in the drug substances.

^c Pyrimidine-2,4(1H,3H)-dione.

^d (2R,5S)-5-(4-Amino-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylic acid (2R,5S)-5-(cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.

^e 1-[(2R,3S,5SS)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine S-oxide.

^f 1-[(2R,3R,5SS)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine S-oxide.

^g 5-Methylpyrimidine-2,4(1H,3H)-dione.

^h 1-[(2S,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

ⁱ 1-(2-Deoxy-β-D-ribofuranosyl)thymine.

^j (2RS,5SR)1-[(2R,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil.

^k 2-Hydroxybenzoic acid.

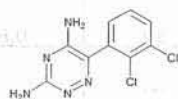
^l 3'-Chloro-3'-deoxythymidine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light, and store between 2° and 30°.

- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
 - USP Lamivudine RS
 - USP Lamivudine Resolution Mixture B RS
 - USP Zidovudine RS

Lamotrigine



$C_9H_7Cl_2N_5$ 256.09
1,2,4-Triazine-3,5-diamine, 6-(2,3-dichlorophenyl);
3,5-Diamino-6-(2,3-dichlorophenyl)-*as*-triazine [84057-84-1].

DEFINITION

Lamotrigine contains NLT 98.0% and NMT 102.0% of $C_9H_7Cl_2N_5$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Diluent: Dilute 8.5 mL of hydrochloric acid with water to 1 L (0.1 M hydrochloric acid).
Buffer: 2.7 g/L of monobasic potassium phosphate in water
Solution A: Triethylamine and *Buffer* (1:150). Adjust with phosphoric acid to a pH of 2.0.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	76.5	23.5
4	76.5	23.5
14	20	80
15	76.5	23.5
19	76.5	23.5

Standard solution: 0.2 mg/mL of USP Lamotrigine RS prepared as follows. Transfer the required amount of USP Lamotrigine RS to a suitable volumetric flask, and add 5% of the final volume with methanol to facilitate dissolution. Dilute with *Diluent* to volume.

Sample solution: 0.2 mg/mL of Lamotrigine prepared as follows. Transfer the required amount of lamotrigine to a suitable volumetric flask, and add 5% of the final volume with methanol to facilitate dissolution. Dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection size: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lamotrigine ($C_9H_7Cl_2N_5$) in the portion of Lamotrigine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)

C_U = concentration of Lamotrigine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II (231):** 10 ppm (Official 1-Jan-2018)

LIMIT OF LAMOTRIGINE RELATED COMPOUND B

Diluent, Solution A, and Sample solution: Prepare as directed in the *Assay*.

Mobile phase: Acetonitrile and *Solution A* (35:65)

System suitability stock solution: 0.2 mg/mL of USP Lamotrigine RS prepared as follows. Transfer the required amount of USP Lamotrigine RS to a suitable volumetric flask, and add 5% of the final volume with methanol to facilitate dissolution. Dilute with *Diluent* to volume.

Standard stock solution: 0.01 mg/mL of USP Lamotrigine Related Compound B RS prepared as follows. Transfer the required amount of USP Lamotrigine Related Compound B RS to a volumetric flask. Add 80% of the flask volume of methanol, and acidify with 1% of the flask volume of hydrochloric acid. Allow to cool, and dilute with methanol to volume. Dilute a portion of this solution with *Diluent*.

System suitability solution: 1 μg/mL of lamotrigine related compound B from the *Standard stock solution* in *System suitability stock solution*

Standard solution: 5 μg/mL of lamotrigine related compound B from the *Standard stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection size: 10 μL

Run time: 2 times the retention time of lamotrigine related compound B

System suitability

Sample: *System suitability solution*

[NOTE—Identify the peaks in the *System suitability solution*, taking into account that lamotrigine is unretained, eluting at or near the solvent front.]

Suitability requirements

Tailing factor: NMT 2.0 for the lamotrigine related compound B peak

Relative standard deviation: NMT 5.0% for the lamotrigine related compound B peak

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lamotrigine related compound B in the portion of Lamotrigine taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- r_u = peak response for lamotrigine related compound B from the *Sample solution*
 r_s = peak response for the lamotrigine related compound B from the *Standard solution*
 C_s = concentration of USP Lamotrigine Related Compound B RS in the *Standard solution* ($\mu\text{g/mL}$)
 C_u = concentration of Lamotrigine in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: NMT 0.1% of lamotrigine related compound B. [NOTE—Lamotrigine related compound D, if present, will elute at a retention time of about 1.5 relative to lamotrigine related compound B. Disregard this peak as it is quantified in the test for *Organic Impurities*.]

• ORGANIC IMPURITIES

Diluent, Buffer, Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability stock solution: 0.2 mg/mL of USP Lamotrigine RS prepared as follows. Transfer the required amount of USP Lamotrigine RS to a suitable volumetric flask, and add 5% of the final volume with methanol to facilitate dissolution. Dilute with *Diluent* to volume.

Impurities stock solution: 0.1 mg/mL of each of USP Lamotrigine Related Compound C RS and USP Lamotrigine Related Compound D RS prepared as follows. Transfer suitable quantities of the Reference Standards to a suitable volumetric flask. Add an amount of methanol equal to 80% of the flask volume, and acidify with 1% of the flask volume of hydrochloric acid. Allow to cool. Dilute with methanol to volume.

System suitability solution: 0.5 $\mu\text{g/mL}$ each of lamotrigine related compound C and lamotrigine related compound D from *Impurities stock solution* in *System suitability stock solution*.

System suitability

Sample: *System suitability solution*

[NOTE—Refer to *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between lamotrigine and lamotrigine related compound C peaks

Analysis

Samples: *Diluent* and *Sample solution*

[NOTE—Disregard any peak that may be present in the chromatogram of the *Diluent* injection. Disregard any peak due to lamotrigine related compound B, because it is quantified in the test for *Limit of Lamotrigine Related Compound B*.]

Calculate the percentage of each impurity in the portion of Lamotrigine taken:

$$\text{Result} = (r_u/r_s) \times (1/F) \times 100$$

- r_u = peak response for each impurity from the *Sample solution*
 r_s = peak response for the lamotrigine peak from the *Sample solution*
 F = relative response factor for each impurity from *Table 2*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lamotrigine	1.0	1.0	—
Lamotrigine related compound C ^a	1.5	1.0	0.1
Lamotrigine related compound B ^{b,c}	3.2	—	—
Lamotrigine related compound D ^d	3.7	0.8	0.2
Any individual, unspecified impurity	—	1.0	0.1
Total impurities, excluding lamotrigine related compound B	—	—	0.2

^a 3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4H)-one.

^b 2,3-Dichlorobenzoic acid.

^c Included only for identification.

^d N-[5-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl]-2,3-dichlorobenzamide.

SPECIFIC TESTS

- **Loss on Drying** (731): Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **USP REFERENCE STANDARDS** (11)
 - USP Lamotrigine RS
 - USP Lamotrigine Related Compound B RS
 - 2,3-Dichlorobenzoic acid.
 $\text{C}_7\text{H}_4\text{Cl}_2\text{O}_2$ 191.01
 - USP Lamotrigine Related Compound C RS
 - 3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4H)-one.
 $\text{C}_9\text{H}_6\text{Cl}_2\text{N}_4\text{O}$ 257.08
 - USP Lamotrigine Related Compound D RS
 - N-[5-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl]-2,3-dichlorobenzamide.
 $\text{C}_{16}\text{H}_9\text{Cl}_4\text{N}_5\text{O}$ 429.09

Lamotrigine Tablets

DEFINITION

Lamotrigine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lamotrigine ($\text{C}_9\text{H}_7\text{Cl}_2\text{N}_3$).

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION** (197U)

Standard solution: 0.02 mg/mL of USP Lamotrigine RS in 0.01 N hydrochloric acid

Sample solution: 0.02 mg/mL of lamotrigine from crushed powdered Tablets in 0.01 N hydrochloric acid

Acceptance criteria: The spectra of the *Standard solution* and *Sample solution* exhibit maxima and minima at the same wavelengths.

- **B.** The retention time of the lamotrigine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Buffer: 0.8 g/L of ammonium acetate. Adjust with glacial acetic acid to a pH of 4.5.

Mobile phase: Methanol and *Buffer* (60:40)

Standard solution: 0.05 mg/mL of USP Lamotrigine RS in *Mobile phase*

Sample solution: Transfer an amount equivalent to 100 mg of lamotrigine from a portion of crushed Tablets (NLT 20) to a suitable volumetric flask to obtain a nominal concentration of lamotrigine of 1.0 mg/mL. Dissolve in 70% of the flask volume of *Mobile phase* by sonicating for 20 min. Dilute with *Mobile phase* to volume. Centrifuge the solution. Quantitatively dilute a suitable volume of centrifugate with *Mobile phase* to obtain a nominal concentration of 0.05 mg/mL of lamotrigine.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection size: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for lamotrigine

Relative standard deviation: NMT 2.0% for lamotrigine

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lamotrigine (C₉H₇Cl₂N₅) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS**• DISSOLUTION (711)****Test 1**

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Determine the amount of lamotrigine (C₉H₇Cl₂N₅) dissolved by using one of the following methods.

Spectrometric method

Standard stock solution: 0.15 mg/mL of USP Lamotrigine RS in *Medium* prepared as follows. Dissolve a suitable quantity in 5% of the flask volume of methanol, then dilute with *Medium* to volume.

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of 0.028 mg/mL.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size. Dilute with *Medium* according to *Table 1*.

Table 1

Tablet Label Claim (mg)	Volume of Sample (mL)	Volume of Volumetric Flask	Final Concentration (mg/mL)
25	—	—	0.028
100	5.0	20	0.029
150	4.0	25	0.027
200	3.0	25	0.027

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 267 nm

Blank: *Medium*

Analysis

Calculate the percentage of the labeled amount of lamotrigine (C₉H₇Cl₂N₅) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

D = dilution factor of the *Sample solution*

V = volume of *Medium*, 900 mL

Chromatographic method

Buffer and Mobile phase: Prepare as directed in the *Assay*.

Standard stock solution: 0.5 mg/mL of USP Lamotrigine RS in *Medium*, prepared as follows. Dissolve a suitable quantity in 15% of the flask volume of methanol, then dilute with *Medium* to volume.

Standard solution: (L/1000) mg/mL of USP Lamotrigine RS from the *Standard stock solution* in *Medium*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Column: 4.6-mm × 15-cm; 5-μm packing L1

Detector: UV 310 nm

Flow rate: 1 mL/min

Injection size: See *Table 2*.

Table 2

Label Claim (mg/Tablet)	Injection Size (μL)
25	50
100, 150, 200	10

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for lamotrigine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lamotrigine (C₉H₇Cl₂N₅) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of lamotrigine is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Apparatus, and Time: Proceed as directed for *Test 1*.

Analysis: Determine the amount of lamotrigine dissolved using either the *Spectrometric method* or *Chromatographic method* described in *Test 1*.

Tolerances: NLT 75% (Q) of the labeled amount of lamotrigine is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 15 min

Standard solution: (L/900) mg/mL of USP Lamotrigine RS in *Medium*, where L is the Tablet label claim in mg

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 270 nm

Cell

For Tablets labeled to contain 100, 150, or

200 mg: 0.2-cm flow cell

For Tablets labeled to contain 25 mg: 1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of lamotrigine is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Buffer: Prepare as directed in the *Assay*.

Mobile phase: Acetonitrile, methanol, and *Buffer* (10:30:60)

Diluent: Methanol and *Buffer* (60:40)

System suitability solution: 1 μ g/mL of Lamotrigine Related Compound B RS and 0.4 mg/mL of USP Lamotrigine RS in *Diluent*

Standard solution: 1.0 μ g/mL of USP Lamotrigine RS in *Diluent*

Sample solution: Transfer an amount equivalent to 100 mg of lamotrigine from a portion of crushed Tablets (NLT 20) to a suitable volumetric flask to obtain a nominal concentration of lamotrigine of about 0.4 mg/mL. Dissolve in 70% of the flask volume of *Mobile phase* by sonicating and shaking intermittently for 30 min. Dilute with *Diluent* to volume. Pass through a membrane filter of 0.45- μ m pore size.

Chromatographic system
(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection size: 5 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between lamotrigine related compound B and lamotrigine, *System suitability solution*

Tailing factor: NMT 2.0 for lamotrigine, *Standard solution*

Relative standard deviation: NMT 10.0% for lamotrigine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of lamotrigine from the *Standard solution*

C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

F = relative response factor for the corresponding impurity (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lamotrigine related compound B ^a	0.67	0.75	0.2
Lamotrigine	1.0	—	—
Lamotrigine related compound C ^b	1.5	1.0	0.5
Any individual unspecified degradation impurity	—	1.0	0.2
Total impurities	—	—	0.75

^a 2,3-Dichlorobenzoic acid.

^b 3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4H)-one.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)
 - USP Lamotrigine RS
 - USP Lamotrigine Related Compound B RS
 - 2,3-Dichlorobenzoic acid.
 - $C_7H_4Cl_2O_2$ 191.01

Lamotrigine Extended-Release Tablets

DEFINITION

Lamotrigine Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile, water, and trifluoroacetic acid (25: 75: 0.05)

Diluent: Acetonitrile, methanol, and water (10:20:70)

Standard solution: 0.25 mg/mL of USP Lamotrigine RS in *Diluent*. Sonication may be used to aid dissolution.

Sample stock solution: 1.0–3.0 mg/mL of lamotrigine prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask containing 10% of the flask volume of

acetonitrile. Allow the Tablets to disperse. Add 20% of the flask volume of methanol. Sonicate for 10 min. Add 30% of the flask volume of 0.1 M hydrochloric acid. Sonicate for 25 min or until a fine, even dispersion is obtained. Allow to cool to room temperature. Dilute with 0.1 M hydrochloric acid to volume. Pass a portion of the solution through a nylon filter of 0.45- μ m pore size and use the filtrate.

Sample solution: Nominally 0.2–0.3 mg/mL of lamotrigine in 0.1 M hydrochloric acid from a suitable volume of *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm \times 15-cm; 3- μ m packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 5 μ L

Run time: NLT 8 times the retention time of lamotrigine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium 1: 0.01 M hydrochloric acid; 700 mL

Medium 2: 7.8 g of tribasic sodium phosphate, and 22.5 g of sodium dodecyl sulfate in 1 L of water. This solution has a pH of about 12.

Apparatus 2: 50 rpm with sinkers (see *Figure 2a* in (711))

Times

For Tablets labeled to contain 25 or 50 mg: 2, 7, 15 h

For Tablets labeled to contain 100, 200, or 250 mg: 2, 5, 12 h

For Tablets labeled to contain 300 mg: 2, 6, 13 h

Procedure: Run the test with *Medium 1* for 2 h. Add 200 mL of *Medium 2*, preheated at 37°. Within 5 min of the addition of *Medium 2*, withdraw the sample for the 2 h time point. Continue the testing by drawing samples at the time points specified in *Table 1*, *Table 2*, or *Table 3*, depending on the label claim.

Diluent: *Medium 1* and *Medium 2* (70:20)

Standard solution: (L/900) mg/mL of USP Lamotrigine RS in *Diluent*, where L is the label claim in mg/Tablet

Sample solution: Pass a suitable portion of the solution under test through a suitable filter of 0.45- μ m pore size. Dilute with *Diluent* if necessary.

Blank: *Diluent*

Instrumental conditions

Mode: UV

Analytical wavelength: 260 nm. [NOTE—Depending on the label claim, cells with suitable path lengths may be used.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount (Q) of lamotrigine ($C_9H_7Cl_2N_3$) dissolved at each time point *i*.

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

D = dilution factor if needed

L = label claim (mg/Tablet)

Tolerances

For Tablets with 25- or 50-mg label claim: See *Table 1*.

For Tablets with 100-, 200-, or 250-mg label claim: See *Table 2*.

For Tablets with 300-mg label claim: See *Table 3*.

Table 1

Time Point (i)	Time (h)	Amount Dissolved
1	2	NMT 10%
2	7	35%–55%
3	15	NLT 80%

Table 2

Time Point (i)	Time (h)	Amount Dissolved	
		100 mg, 200 mg	250 mg
1	2	NMT 10%	NMT 10%
2	5	20%–45%	20%–40%
3	12	NLT 80%	NLT 80%

Table 3

Time Point (i)	Time (h)	Amount Dissolved
1	2	NMT 10%
2	6	25%–45%
3	13	NLT 80%

The percentages of the labeled amount of lamotrigine ($C_9H_7Cl_2N_3$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* (711).

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Diluent 1: Acetonitrile, methanol, and 0.1 M hydrochloric acid (10:20:70)

Diluent 2: Acetonitrile, methanol, and water (10:20:70)

System suitability stock solution: 0.025 mg/mL of USP Lamotrigine Related Compound C RS in *Diluent 1*

System suitability solution: 1.25 μ g/mL of USP Lamotrigine Related Compound C RS and 0.25 mg/mL of USP Lamotrigine RS in *Diluent 2* prepared as follows. Transfer a suitable amount of USP Lamotrigine RS to a suitable volumetric flask. Transfer a suitable volume of *System suitability stock solution* to the flask. Dissolve and dilute with *Diluent 2* to volume.

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 10 between the lamotrigine and lamotrigine related compound C peaks

Signal-to-noise ratio: NLT 100 for lamotrigine related compound C

Analysis

Sample: *Sample solution*

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each impurity from the *Sample solution*

r_T = sum of all of the impurity peak responses and the lamotrigine peak response from the *Sample solution*

Acceptance criteria: See Table 4. Disregard peaks less than 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lamotrigine	1.0	—
Lamotrigine related compound C	1.7	0.3
Lamotrigine dimer ^a	6.0	0.2
Any individual unspecified degradation product	—	0.2
Total impurities	—	0.5

^aThis is either lamotrigine o-dimer [N^3,N^5 -methylenebis(6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine)] or lamotrigine-dimer N^3,N^5 -methylenebis(6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
 - USP Lamotrigine RS
 - USP Lamotrigine Related Compound C RS
 - 3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4H)-one.
 - $C_9H_6Cl_2N_4O$ 257.08

Lamotrigine Tablets for Oral Suspension

DEFINITION

Lamotrigine Tablets for Oral Suspension contain NLT 90.0% and NMT 110.0% of the labeled amount of lamotrigine ($C_9H_7Cl_2N_3$).

IDENTIFICATION

A. INFRARED ABSORPTION (197K)

Sample: Transfer crushed powder of the Tablets for Oral Suspension, equivalent to 30 mg of lamotrigine, into an Erlenmeyer flask, and add 10 mL of chloroform. Sonicate for about 15 min. Shake the flask for another 2 min. Pass the sample through a Whatman No. 1 filter paper. Evaporate the solution to dryness. Add 250 mg of potassium bromide to the dried residue, and prepare the pellet.

Acceptance criteria: Absorption bands at 1491 cm^{-1} , 1557 cm^{-1} , 1621 cm^{-1} , 3213 cm^{-1} , 3320 cm^{-1} , and 3451 cm^{-1} are found in the *Sample* and a similarly prepared Standard.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Buffer: 0.77 g/L of ammonium acetate, adjusted with glacial acetic acid to a pH of 4.5

Mobile phase: Acetonitrile, methanol, and Buffer (30:10:60)

Diluent: Acetonitrile, methanol, and Buffer (30:30:40)

Standard solution: 0.05 mg/mL of USP Lamotrigine RS in Diluent

Sample solution: Transfer NLT 6 Tablets for Oral Suspension to a suitable volumetric flask to obtain a nominal concentration of about 0.05 mg/mL of lamotrigine. Sonicate in 70% of the flask volume of Diluent for 30 min with intermittent shaking. Dilute with Diluent to final volume, and pass a portion through a suitable membrane filter.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: 210 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1.5 mL/min

Injection size: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0, lamotrigine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lamotrigine ($C_9H_7Cl_2N_3$), based on the label claim, in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL, degassed

Apparatus 2: 50 rpm

Time: 15 min

[NOTE—The *Sample solution* may be analyzed using either Chromatographic procedure 1 or Chromatographic procedure 2.]

Standard stock solution: 0.5 mg/mL of USP Lamotrigine RS in methanol

Standard solution: ($L/1000$) mg/mL of USP Lamotrigine RS in Medium from the *Standard stock solution*, where L is the Tablet label claim in mg

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Determine the amount of lamotrigine dissolved by employing one of the following chromatographic procedures.

Chromatographic procedure 1

Buffer: To 1 L of 0.77 g/L of ammonium acetate in water add 2 mL of triethylamine, and adjust with glacial acetic acid to a pH of 7.5.

Mobile phase: Acetonitrile, methanol, and Buffer (20:15:65)

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 310 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection size: 100 μL

System suitabilitySample: *Standard solution***Suitability requirements**

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Chromatographic procedure 2**Mobile phase and Chromatographic system:** Proceed as directed in the test for *Organic Impurities, Procedure 2*.**System suitability**Sample: *Standard solution***Suitability requirements**

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of lamotrigine dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL) L = label claim (mg/Tablet) V = volume of *Medium*, 900 mL**Tolerances:** NLT 80% (Q) of the labeled amount of lamotrigine is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES**Organic Impurities**

[NOTE—*Procedure 1* is recommended if lamotrigine related compound B is a potential organic impurity. *Procedure 2* is recommended if lamotrigine related compound C is a potential organic impurity.]

• **PROCEDURE 1****Buffer, Mobile phase, and Diluent:** Prepare as directed in the *Assay*.**Standard solution:** 0.8 μg/mL of USP Lamotrigine RS in *Diluent***Sample solution:** From NLT 20 Tablets for Oral Suspension ground to a fine powder, transfer an amount of powder to a suitable flask, to obtain a nominal concentration of 0.25 mg/mL of lamotrigine in *Diluent*. Sonicate for 15 min to dissolve the contents. Filter a portion, and discard the first 1 mL of the filtrate.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm column; 5-μm packing L1

Flow rate: 1 mL/min

Injection size: 20 μL

System suitabilitySample: *Standard solution*[NOTE—Relative retention times are in *Table 1*.]**Suitability requirements**

Tailing factor: NMT 2.0

Relative standard deviation: NMT 10%

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak response of the impurity from the *Sample solution* r_S = peak response of lamotrigine from the *Standard solution* C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL) C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL) F = relative response factor for each impurity listed in *Table 1***Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lamotrigine	1.0	—	—
Lamotrigine related compound B ^a	1.59	0.69	0.1
Any other individual degradation product	—	1.0	0.2
Total impurities	—	—	0.4

^a 2,3-Dichlorobenzoic acid.• **PROCEDURE 2****Mobile phase:** Acetonitrile, water, glacial acetic acid, and triethylamine (47:148:4:1). [NOTE—The *Mobile phase* is stable for 48 h at room temperature.]**Diluent:** Methanol and water (40:60)**Standard solution:** 0.2 mg/mL of USP Lamotrigine RS and 0.002 mg/mL of USP Lamotrigine Related Compound C RS prepared as follows. Transfer suitable amounts of USP Lamotrigine RS and USP Lamotrigine Related Compound C RS to a suitable volumetric flask. Add 40% of the flask volume of methanol, and sonicate until dissolved. Allow to cool to room temperature, and dilute with water to volume.**Sample solution:** Nominally 0.2 mg/mL of lamotrigine. Use 10 Tablets for Oral Suspension for a label claim of 25 mg or less and 5 Tablets for Oral Suspension for a label claim of 50 mg or more prepared as follows. Transfer the appropriate number of Tablets for Oral Suspension to a suitable volumetric flask. Add 40% of the flask volume of water. Swirl until the Tablets have disintegrated. Allow the effervescence to stop, and then add an additional 40% of the flask volume of methanol. Sonicate the flask for 10 min, and cool to room temperature. Dilute with water to volume. [NOTE—For Tablets for Oral Suspension with a 50 mg or higher label claim, a suitable intermediate concentration may be chosen. The final dilution to arrive at the nominal concentration is made using *Diluent*.]**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 15-cm column; 5-μm packing L1

Flow rate: 1 mL/min

Injection size: 10 μL

System suitabilitySample: *Standard solution*[NOTE—Relative retention times are in *Table 2*.]**Suitability requirements****Resolution:** NLT 2.0 between lamotrigine and lamotrigine related compound C**Tailing factor:** NMT 2.0 for lamotrigine and lamotrigine related compound C**Relative standard deviation:** NMT 5.0% for lamotrigine related compound C and NMT 1.5% for lamotrigine

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lamotrigine related compound C in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of lamotrigine related compound C from the *Sample solution*
 r_S = peak response of lamotrigine related compound C from the *Standard solution*
 C_S = concentration of USP Lamotrigine Related Compound C RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of any other impurity from the *Sample solution*
 r_S = peak response of lamotrigine from the *Standard solution*
 C_S = concentration of USP Lamotrigine RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lamotrigine	1.0	—
Lamotrigine related compound C ^a	1.3	0.3
Any other individual unspecified degradation product	—	0.2
Total impurities	—	0.5

^a 3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4H)-one.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in tight, light-resistant containers, at controlled room temperature.
- **LABELING:** If a procedure for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* procedure the article complies. The label states that the Tablets for Oral Suspension may be swallowed whole, chewed, or dispersed in water or diluted fruit juice.
- **USP REFERENCE STANDARDS (11)**
 - USP Lamotrigine RS
 - 1,2,4-Triazine-3,5-diamine, 6-(2,3-dichlorophenyl). $C_9H_7Cl_2N_5$ 256.09
 - USP Lamotrigine Related Compound C RS
 - 3-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4H)-one. $C_9H_6Cl_2N_4O$ 257.08

Lamotrigine Compounded Oral Suspension

DEFINITION

Lamotrigine Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$).

Prepare Lamotrigine Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Lamotrigine tablet ^a equivalent to	100 mg
Ora-Blend, ^b a sufficient quantity to make	100 mL

^a Lamotrigine 100-mg tablet, Torrent Pharmaceuticals LTD, Kalamazoo, MI.

^b Perrigo, Minneapolis, MN.

Place the required number of tablet(s) in a suitable mortar, and comminute to a fine powder. Add the *Ora-Blend* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Ora-Blend* to make a lamotrigine liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Ora-Blend* to bring to final volume, and mix well.

ASSAY• **PROCEDURE**

Solution A: Dissolve 2.7 g of monobasic potassium phosphate in 1000 mL of water. Add 6.5 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.0.

Mobile phase: Acetonitrile and *Solution A* (20:80). Filter, and degas.

Diluent: 0.1 M hydrochloric acid

Standard solution: 0.4 mg/mL of USP Lamotrigine RS in *Diluent*

Sample solution: Shake thoroughly each bottle of Oral Suspension. Transfer 4 mL of Oral Suspension into a 10 mL volumetric flask, dilute with *Diluent* to volume, and mix well.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.0 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for lamotrigine is about 9.8 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lamotrigine ($C_9H_7Cl_2N_5$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lamotrigine from the *Sample solution*

r_S = peak response of lamotrigine from the *Standard solution*

C_S = concentration of lamotrigine in the *Standard solution* (mg/mL)

C_U = nominal concentration of lamotrigine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH (791):** 4.0–5.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or 2°–8°.
- **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*.

- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature or 2°–8°
- **USP REFERENCE STANDARDS (11)**
USP Lamotrigine RS

Lanolin

DEFINITION

Lanolin is the purified, wax-like substance from the wool of sheep, *Ovis aries* L. (Fam. Bovidae), that has been cleaned, decolorized, and deodorized. It contains NMT 0.25% of water. It may contain NMT 0.02% of a suitable antioxidant.

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%
- **CHLORIDE AND SULFATE, Chloride (221)**

Sample solution: Boil 20 mL of alcohol with 1.0 g of Lanolin under a reflux condenser. Cool, add 1 mL of 2 N nitric acid, and filter. To the filtrate add 5 drops of a solution of 20 mg/mL of silver nitrate in alcohol.

Blank: Boil 20 mL of alcohol under a reflux condenser. Cool, add 1 mL of 2 N nitric acid, and filter. To the filtrate add 5 drops of a solution of 20 mg/mL of silver nitrate in alcohol. Add 0.50 mL of 0.020 N hydrochloric acid.

Acceptance criteria: 0.035%; any turbidity produced by the *Sample solution* does not exceed that produced by the *Blank*.

FOREIGN SUBSTANCES

Use pesticide-free grade reagents and solvents throughout this test. [NOTE—Reference materials of pesticides for use in the *Standard solution* may be obtained from any commercial source.¹]

Standard stock solutions: Prepare stock solutions for each reference pesticide containing 100 mg/L in hexane.

[NOTE—Concentrated stock solutions may be stored in glass-stoppered containers in a dark refrigerator at 2°–5° for up to 1 year. Most pesticides may be dissolved directly in hexane; however, the hexachlorocyclohexane isomers and the DDT group of pesticides may require initial dissolution in the minimum volume of acetone followed by dilution with hexane to the specified concentration.]

Standard solution: Dilute volumes of the *Standard stock solutions* quantitatively with hexane, and combine to obtain a composite *Standard solution* having the concentrations indicated in Table 1. Store the composite *Standard solution* in a glass-stoppered glass container in the dark at 2°–5°, and replace it every 2 months.

[NOTE—Two or more separate composite *Standard solutions*, each preferably containing NMT 8 reference pesticides, may be prepared if needed. Reference pesticides should be selected for composite *Standard solutions* on the basis that relative retention times (see Table 1) differ sufficiently so that peaks in chromatograms will not be expected to overlap, and they should be selected and combined appropriately for the chromatographic system and detector used.]

¹ Suitable materials may be obtained from either Chem Service, 660 Tower Lane, P.O. Box 3108, Westchester, PA 19381-3108 or Greyhound, 88 Grange Road West, Birkenhead, Merseyside, L43 4XF, England U.K.

Table 1

Reference Pesticide ^a	Standard Solution (Concentration in µg/mL)		Relative Retention Times (Relative to 1.0 for Chlorpyrifos)	
	Electron-Capture Detector	Flame-Photometric Detector	System I	System II
Tetrachloronitrobenzene (TCBN)	0.05	—	0.29	0.24
alpha-Hexachlorocyclohexane (alpha BHC)	0.05	—	0.40	0.35
beta-Hexachlorocyclohexane (beta BHC)	0.30	—	0.43	0.56
Hexachlorobenzene (HCB)	0.05	—	0.45	0.33
gamma-Hexachlorocyclohexane (lindane)	0.05	—	0.48	0.41
Propetamphos	—	0.30	0.48	0.42
Diazinon	—	0.20	0.52	0.40
Dichlofenthion	0.10	0.20	0.67	0.56
Ronnel	0.30	0.40	0.81	0.66
Heptachlor	0.10	—	0.83	0.60
Malathion	—	0.40	0.91	1.05
Chlorpyrifos	0.30	0.30	1.00	1.00
Aldrin	0.20	—	1.05	0.76
Pirimiphos ethyl	—	0.40	1.14	1.14
Chlorfenvinphos Z	0.40	0.40	1.17	1.40
Heptachlor epoxide	0.20	—	1.29	1.17
Chlorfenvinphos E	0.40	0.50	1.30	1.51
Bromophos ethyl	0.40	0.50	1.51	1.45
1,1'-Dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethene (o,pp-DDE)	0.30	—	1.55	1.51
1,1'-Dichloro-2-(4-chlorophenyl)-2-(4-chlorophenyl)ethene (p,pp-DDE)	0.30	—	1.88	1.86
Stirophos	0.60	0.80	1.58	1.97
alpha-Endosulfan	0.40	—	1.63	1.47
1,1'-Dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (o,pp-TDE)	0.40	—	1.90	2.19

^a Suitable materials may be obtained from either Chem Service, 660 Tower Lane, P.O. Box 3108, Westchester, PA 19381-3108 or Greyhound, 88 Grange Road West, Birkenhead, Merseyside, L43 4XF, England U.K.

Table 1 (Continued)

Reference Pesticide ^a	Standard Solution (Concentration in µg/mL)		Relative Retention Times (Relative to 1.0 for Chlorpyrifos)	
	Electron-Capture Detector	Flame-Photometric Detector	System I	System II
Dieldrin	0.30	—	1.91	1.84
Endrin	0.40	—	2.13	2.29
beta-Endosulfan	0.40	—	2.19	2.77
1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane (<i>p,p'</i> -TDE)	0.40	—	2.41	2.87
1,1,1-Trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (<i>o,p,p'</i> -DDT)	0.40	—	2.55	2.70
Ethion	1.00	0.40	2.56	3.36
Carbophenothion	0.80	1.00	2.94	3.70
1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (<i>p,p'</i> -DDT)	0.50	—	3.13	3.50
Methoxychlor	0.60	—	4.70	7.20
Carbophenothion sulfone	5.00	—	5.10	9.20
Carbophenothion sulfoxide	5.00	—	5.40	10.00

^a Suitable materials may be obtained from either Chem Service, 660 Tower Lane, P.O. Box 3108, Westchester, PA 19381-3108 or Greyhound, 88 Grange Road West, Birkenhead, Merseyside, L43 4XF, England U.K.

Gel permeation chromatography cleanup system

Eluant: Methylene chloride and hexane (1:1)

Column: 25-mm × 50-cm; packed with a slurry of 35 g of styrene-divinylbenzene copolymer beads compressed to a bed length of about 20 cm

Operating pressure: 8–11 psi

Flow rate: 5 mL/min

Set up the chromatograph, adjusting to discard the fraction eluting from 0 to 12 min. Collect the fraction eluting from 12 to 32 min, and rinse for 2 min, discarding the rinse fraction.

System suitability

Elution of lanolin: Melt a suitable quantity of Lanolin, and pass through a fluted filter paper into a container. Transfer 6.0 g to a 50-mL volumetric flask. Dilute with *Eluant* to volume, and filter. Transfer 5.0 mL of this solution to the gel permeation chromatographic column, and elute with *Eluant*. Collect 100 mL of the column effluent in tared beakers in 10-mL increments. Evaporate the solvent, cool, weigh the beakers and contents, and calculate the amount of lanolin eluted in each 10-mL increment. The column is suitable if NLT 96% of the lanolin elutes in the first 60 mL.

Elution of pesticide from lanolin: Dissolve suitable quantities of diazinon, diclofenthion, bromophos ethyl, lindane, and dieldrin in hexane to obtain a *Standard solution* having concentrations of 0.4, 0.4, 1.0, 0.1, and 0.6 µg/mL, respectively. Transfer 5.0 mL of this solution to a 10-mL volumetric flask containing 1 g of USP Lanolin RS. Dilute with methylene chloride to volume. Transfer 5 mL of this solution to the gel permeation chromatographic column, and elute with 160 mL of *Eluant*. Discard the first 60-mL fraction, and collect the next 100-mL fraction (from 60 to 160 mL). Transfer this collection fraction to a concentrator fitted with a graduated collection flask, add 50 mL of hexane, and concentrate by evaporation to 5 mL. Inject this fraction into the chromatographs described in *Chromatographic system I* and *Chromatographic system II*. Record the chromatograms, and measure the heights of the peaks obtained from the five pesticides in the *Standard solution*. Calculate the recoveries of each of the five pesticides used in the fortified USP Lanolin RS solution.

Prepare a test solution by mixing hexane with the *Standard solution* (1:1). Inject this into the chromatographs described in *Chromatographic system I* and *Chromatographic system II*. Record the chromat-

ograms, and measure the peak heights of the five pesticides in the chromatogram of the *Sample solution*. Compare the peak heights from the fraction of the *Standard solution* to the peak heights of the corresponding pesticides from the *Sample solution*: NLT 85% of the added amounts of each of the five pesticides is recovered.

Sample solution: Transfer 6 g of Lanolin, previously melted to liquid form by heating on a hot water bath if necessary, to a 50-mL volumetric flask. Dissolve in 25 mL of *Eluant*, dilute with *Eluant* to volume, and filter. Transfer 5.0 mL of this solution to the column, and elute with 160 mL of *Eluant*. Discard the first 60-mL fraction, and collect the remaining fraction in a suitable evaporator. Concentrate by evaporation on a steam bath to 3 mL, add 50 mL of hexane, and evaporate again to remove all traces of methylene chloride, adjusting the volume with hexane to 3.0 mL.

Chromatographic system I

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Electron capture

Column: 0.53-mm × 30-m fused silica capillary; bonded with a 1.5-µm layer of phase G1, and a 0.53-mm × 6-m fused silica uncoated guard column connected to a modified packed column-type injector system

Column temperature: 200°. [NOTE—The initial temperature of the column may be adjusted so that the retention times of ethion and *p,p'*-DDT are 2.56 and 3.1, respectively, relative to chlorpyrifos.]

Carrier gas: Helium

Flow rate: 25 mL/min. Adjust so that the retention time of chlorpyrifos is 4 min.

Makeup gas: Nitrogen, 40 mL per minute

Injection volume: 5 µL

Chromatographic system II

Mode: GC

Detector: Flame photometric

Column: 0.53-mm × 30-m fused silica capillary; bonded with a 1.0-µm layer of phase G3, and a 0.53-mm × 6-m fused silica uncoated guard column connected to a modified packed column-type injector system

Column temperature: 200°. [NOTE—The initial temperature of the column may be adjusted so that the retention time of ethion is 3.36 relative to that of chlorpyrifos.]

Carrier gas: Helium
 Flow rate: 25 mL/min. Adjust so that the retention time of chlorpyrifos is 4 min.
 Makeup gas: Nitrogen, 40 mL/min
 Injection volume: 5 μ L

Analysis

The following procedure is to be followed for *Chromatographic systems I and II*.

Samples: *Standard solution* and *Sample solution*
 Calculate the quantity of the individual specified residue found in the sample taken:

$$\text{Result} = (r_u/r_s) \times (C/W) \times 30$$

r_u = peak area of each residue from the *Sample solution*
 r_s = peak area of each residue from the *Standard solution*
 C = concentration of the reference pesticide in the *Standard solution* (mg/L)
 W = weight of Lanolin taken (g)

Acceptance criteria

Individual specified residue: NMT 10 ppm
 Total specified residue: NMT 40 ppm

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE, Class II (741)**
Analysis: Determine on a sample previously cooled to 8°–10°.
Acceptance criteria: 38°–44°
- **FATS AND FIXED OILS, Acid Value (Free Fatty Acids) (401)**
Sample: 10.0 g
Acceptance criteria: The free acids obtained from the *Sample* require NMT 2.0 mL of 0.10 N sodium hydroxide for neutralization.
- **FATS AND FIXED OILS, Iodine Value (401)**
Sample: 780–820 mg
Acceptance criteria: 18–36
- **ALKALINITY**
Sample: 2.0 g
Analysis: Dissolve the *Sample* in 10 mL of ether, and add 2 drops of phenolphthalein TS.
Acceptance criteria: The liquid is not colored red.
- **WATER-SOLUBLE ACIDS AND ALKALIES**
Sample: 10.0 g
Analysis: Warm the *Sample* with 50 mL of water on a steam bath, constantly stirring the mixture until the Lanolin is melted.
Acceptance criteria: The fat separates completely on cooling, leaving the water layer nearly clear and neutral to litmus. Retain the water layer for the test for *Water-Soluble Oxidizable Substances* and *Ammonia*.
- **WATER-SOLUBLE OXIDIZABLE SUBSTANCES**
Sample solution: 10 mL of the solution from *Water-Soluble Acids and Alkalies*
Analysis: Add the *Sample solution* to 50 μ L of 0.10 N potassium permanganate.
Acceptance criteria: The resulting solution does not completely decolorize within 10 min.
- **AMMONIA**
Sample solution: 10 mL of the solution from *Water-Soluble Acids and Alkalies*
Analysis: Add 1 mL of 1 N sodium hydroxide to the *Sample solution*, and boil.
Acceptance criteria: The vapors do not turn red litmus to blue.
- **WATER DETERMINATION, Method I (921)**
Solution A: Chloroform and methanol (3:2)
Sample solution: 250 mg/mL of Lanolin in *Solution A*
Analysis: Determine the water content of a 10.0-mL portion of the *Sample solution*. Perform a blank determination on 10.0 mL of *Solution A*, and make any necessary correction.

Acceptance criteria: NMT 0.25%

PETROLATUM

Sample: 3 g

Analysis: Heat the *Sample* on a steam bath, with frequent stirring, until its weight loss is NLT its water content. Boil 40 mL of dehydrated alcohol with 500 mg of the dried lanolin so obtained.

Acceptance criteria: The solution is clear or NMT opalescent.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, preferably at controlled room temperature.
- **LABELING:** The label states that it is not to be used undiluted.
- **USP REFERENCE STANDARDS (11)**
 USP Lanolin RS

Modified Lanolin

DEFINITION

Modified Lanolin is the purified wax-like substance from the wool of sheep, *Ovis aries* L. (Fam. Bovidae), that has been processed to reduce the contents of free lanolin alcohols and residues of detergent and pesticide. It contains NMT 0.25% of water. It may contain NMT 0.02% of a suitable antioxidant.

IMPURITIES

LIMIT OF FREE LANOLIN ALCOHOLS

Gel permeation chromatographic cleanup system

Eluant: Methylene chloride

Column: 25-mm \times 100-cm; packed with a slurry of styrene-divinylbenzene copolymer beads compressed to a bed length of approximately 77 cm

Flow rate: 4 mL/min

Set up the chromatograph, adjusting to discard the fraction eluting from 0 to 43 min. Collect the fraction eluting from 43 to 60 min, and rinse for 20 min, discarding the rinse fraction.

System suitability

Elution of lanolin alcohols: Melt a suitable quantity of USP Lanolin Alcohols RS, and pass through a fluted filter paper into a container. Transfer 1.0 g to a 10-mL volumetric flask. Dilute with *Eluant* to volume. Transfer 5 mL to the gel permeation chromatographic column, and elute with *Eluant*. Collect 172–240 mL of the column effluent in a suitable evaporator. Evaporate the solvent, cool, weigh the evaporator, and calculate the amount of lanolin alcohols eluted in the evaporator. The column is suitable if NLT 99% of the lanolin alcohols elute in the first 172–240 mL.

Standard solution: 0.5 mg/mL of USP Lanolin Alcohols RS in hexane. Store this solution in a cold, dark place for up to 4 weeks. Before using, warm just sufficiently to dissolve any precipitate if necessary.

Sample solution: Transfer 1 g of Modified Lanolin, previously melted to liquid form by heating on a hot water bath if necessary, to a 10-mL volumetric flask. Dissolve in 7 mL of *Eluant*, dilute with *Eluant* to volume, and filter. Transfer 5.0 mL of this solution to the column, and elute with 320 mL of *Eluant*. Discard the first 172-mL fraction, and collect the next 68-mL fraction (from 172 to 240 mL) in a suitable evaporator. Concentrate by evaporation on a steam bath to 3 mL. Add 50 mL of hexane, and transfer this solution to a 100-mL volumetric flask, adjusting the volume with hexane to 100 mL.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Columns

Guard: 0.32-mm × 50-cm fused silica uncoated

Analytical: 0.33-mm × 50-m fused silica capillary;
bonded with a 0.50-μm layer of phase G2**Temperatures**

Detector: 290°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
210	3	280	—

Flow rate: 7 mL/min

Carrier gas: Nitrogen

Makeup gas: Nitrogen at 50 mL/min

Injection volume: 1 μL

Analysis**Samples:** *Standard solution* and *Sample solution*[NOTE—Allow both the *Standard solution* and the *Sample solution* to elute for NLT 40 min.]

Calculate the percentage of free lanolin alcohols in the portion of Modified Lanolin taken:

$$\text{Result} = (r_u/r_s) \times [(C \times K)/(I \times W)] \times 100$$

 r_u = total peak response from the *Sample solution* r_s = total peak response from the *Standard solution* C = concentration of USP Lanolin Alcohols RS in the *Standard solution* (mg/mL) I = volume injected into the gel permeation chromatography column (mL) W = weight of Modified Lanolin taken (g) K = corrected fraction of free lanolin alcohols in the USP Lanolin Alcohols RS in the *Standard solution* taken:

$$K = 1 + (0.0062A - 0.0119S)$$

 A = acid value of USP Lanolin Alcohols RS S = saponification value of USP Lanolin Alcohols RS

Acceptance criteria: NMT 6%

• FOREIGN SUBSTANCESUse pesticide-free grade reagents and solvents throughout this test. [NOTE—Reference materials of pesticides for use in the *Standard solution* may be obtained from any commercial source.¹]**Standard stock solutions:** Prepare stock solutions for each reference pesticide containing 100 mg/L in hexane.

[NOTE—Concentrated stock solutions may be stored in glass-stoppered containers in a dark refrigerator at 2°–5° for up to 1 year. Most pesticides may be dissolved directly in hexane; however, the hexachlorocyclohexane isomers and the DDT group of pesticides may require initial dissolution in the minimum volume of acetone followed by dilution with hexane to the specified concentration.]

Standard solution: Dilute volumes of the *Standard stock solutions* quantitatively with hexane, and combine to obtain a composite *Standard solution* having the concentrations indicated in Table 2. Store the composite *Standard solution* in a glass-stoppered glass container in the dark at 2°–5°, and replace it every 2 months.[NOTE—Two or more separate composite *Standard solutions*, each preferably containing NMT 8 reference pesticides, may be prepared if needed. Reference pesticides should be selected for composite *Standard solutions* on the basis that relative retention times (see Table 2) differ sufficiently so that peaks in chromatograms will not be expected to overlap, and they should be selected and combined appropriately for the chromatographic system and detector used.]¹ Suitable materials may be obtained from either Chem Service, 660 Tower Lane, P.O. Box 3108, Westchester, PA 19381-3108 or Greyhound, 88 Grange Road West, Birkenhead, Merseyside, L43 4XF, England U.K.**Table 2**

Reference Pesticide ^a	Standard Solution (Concentration in μg/mL)		Relative Retention Times (Relative to 1.0 for Chlorpyrifos)	
	Electron-Capture Detector	Flame-Photometric Detector	System I	System II
Tetrachloronitrobenzene (TCBN)	0.05	—	0.29	0.24
alpha-Hexachlorocyclohexane (alpha BHC)	0.05	—	0.40	0.35
beta-Hexachlorocyclohexane (beta BHC)	0.30	—	0.43	0.56
Hexachlorobenzene (HCB)	0.05	—	0.45	0.33
gamma-Hexachlorocyclohexane (lindane)	0.05	—	0.48	0.41
Propetamphos	—	0.30	0.48	0.42
Diazinon	—	0.20	0.52	0.40
Dichlofenthion	0.10	0.20	0.67	0.56
Ronnel	0.30	0.40	0.81	0.66
Heptachlor	0.10	—	0.83	0.60
Malathion	—	0.40	0.91	1.05
Chlorpyrifos	0.30	0.30	1.00	1.00
Aldrin	0.20	—	1.05	0.76
Pirimiphos ethyl	—	0.40	1.14	1.14
Chlorfenvinphos Z	0.40	0.40	1.17	1.40
Heptachlor epoxide	0.20	—	1.29	1.17
Chlorfenvinphos E	0.40	0.50	1.30	1.51

^a Suitable materials may be obtained from either Chem Service, 660 Tower Lane, P.O. Box 3108, Westchester, PA 19381-3108 or Greyhound, 88 Grange Road West, Birkenhead, Merseyside, L43 4XF, England U.K.

Table 2 (Continued)

Reference Pesticide ^a	Standard Solution (Concentration in µg/mL)		Relative Retention Times (Relative to 1.0 for Chlorpyrifos)	
	Electron-Capture Detector	Flame-Photometric Detector	System I	System II
Bromophos ethyl	0.40	0.50	1.51	1.45
1,1-Dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethene (<i>o,p</i> -DDE)	0.30	—	1.55	1.51
1,1-Dichloro-2-(4-chlorophenyl)-2-(4-chlorophenyl)ethene (<i>p,p</i> -DDE)	0.30	—	1.88	1.86
Stirophos	0.60	0.80	1.58	1.97
alpha-Endosulfan	0.40	—	1.63	1.47
1,1-Dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (<i>o,p</i> -TDE)	0.40	—	1.90	2.19
Dieldrin	0.30	—	1.91	1.84
Endrin	0.40	—	2.13	2.29
beta-Endosulfan	0.40	—	2.19	2.77
1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane (<i>p,p</i> -TDE)	0.40	—	2.41	2.87
1,1,1-Trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (<i>o,p</i> -DDT)	0.40	—	2.55	2.70
Ethion	1.00	0.40	2.56	3.36
Carbophenothion	0.80	1.00	2.94	3.70
1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (<i>p,p</i> -DDT)	0.50	—	3.13	3.50
Methoxychlor	0.60	—	4.70	7.20
Carbophenothion sulfone	5.00	—	5.10	9.20
Carbophenothion sulfoxide	5.00	—	5.40	10.00

^a Suitable materials may be obtained from either Chem Service, 660 Tower Lane, P.O. Box 3108, Westchester, PA 19381-3108 or Greyhound, 88 Grange Road West, Birkenhead, Merseyside, L43 4XF, England U.K.

Gel permeation chromatography cleanup system

Eluant: Methylene chloride and hexane (1:1)

Column: 25-mm × 50-cm; packed with a slurry of 35 g of styrene-divinylbenzene copolymer beads compressed to a bed length of about 20 cm

Operating pressure: 8–11 psi

Flow rate: 5 mL/min. Set up the chromatograph, adjusting to discard the fraction eluting from 0 to 12 min. Collect the fraction eluting from 12 to 32 min, and rinse for 2 min, discarding the rinse fraction.

System suitability

Elution of lanolin: Melt a suitable quantity of Lanolin, and pass through a fluted filter paper into a container. Transfer 6.0 g to a 50-mL volumetric flask. Dilute with *Eluant* to volume, and filter. Transfer 5.0 mL of this solution to the gel permeation chromatographic column, and elute with *Eluant*. Collect 100 mL of the column effluent in tared beakers in 10-mL increments. Evaporate the solvent, cool, weigh the beakers and contents, and calculate the amount of lanolin eluted in each 10-mL increment. The column is suitable if NLT 96% of the lanolin elutes in the first 60 mL.

Elution of pesticide from lanolin: Dissolve suitable quantities of diazinon, diclofenthion, bromophos ethyl, lindane, and dieldrin in hexane to obtain a *Standard solution* having concentrations of 0.4, 0.4, 1.0, 0.1, and 0.6 µg/mL, respectively. Transfer 5.0 mL of this solution to a 10-mL volumetric flask containing 1 g of USP Lanolin RS. Dilute with methylene chloride to volume. Transfer 5 mL of this solution to the gel permeation chromatographic column, and elute with 160 mL of *Eluant*. Discard the first 60-mL fraction, and collect the next 100-mL fraction (from 60 to 160 mL). Transfer this collection fraction to a concentrator fitted with a graduated collection flask, add 50 mL of hexane, and concentrate by evaporation to 5 mL. Inject this fraction into the chromatographs described in *Chromatographic system I* and *Chromatographic system II*. Record the chromatograms, and

measure the heights of the peaks obtained from the five pesticides in the *Standard solution*. Calculate the recoveries of each of the five pesticides used in the fortified USP Lanolin RS solution.

Prepare a test solution by mixing hexane with the *Standard solution* (1:1). Inject into the chromatographs described in *Chromatographic system I* and *Chromatographic system II*. Record the chromatograms, and measure the peak heights of the five pesticides in the chromatogram of the *Sample solution*. Compare the peak heights from the fraction of the *Standard solution* to the peak heights of the corresponding pesticides from the *Sample solution*: NLT 85% of the added amounts of each of the five pesticides is recovered.

Sample solution: Transfer 6 g of Lanolin, previously melted to liquid form by heating on a hot water bath if necessary, to a 50-mL volumetric flask. Dissolve in 25 mL of *Eluant*, dilute with *Eluant* to volume, and filter. Transfer 5.0 mL of this solution to the column, and elute with 160 mL of *Eluant*. Discard the first 60-mL fraction, and collect the remaining fraction in a suitable evaporator. Concentrate by evaporation on a steam bath to 3 mL, add 50 mL of hexane, and evaporate again to remove all traces of methylene chloride, adjusting the volume with hexane to 3.0 mL.

Chromatographic system I
(See *Chromatography* <621>, *System Suitability*.)

Mode: GC

Detector: Electron capture

Column: 0.53-mm × 30-m fused silica capillary; bonded with a 1.5-µm layer of phase G1, and a 0.53-mm × 6-m fused silica uncoated guard column connected to a modified packed column-type injector system

Column temperature: 200°. [NOTE—The initial temperature of the column may be adjusted so that the retention times of ethion and *p,p'*-DDT are 2.56 and 3.1, respectively, relative to chlorpyrifos.]

Carrier gas: Helium

Flow rate: 25 mL/min. Adjust so that the retention time of chlorpyrifos is 4 min.

Makeup gas: Nitrogen, 40 mL/min

Injection volume: 5 μ L

Chromatographic system II

Mode: GC

Detector: Flame photometric

Column: 0.53-mm \times 30-m fused silica capillary; bonded with a 1.0- μ m layer of phase G3, and a 0.53-mm \times 6-m fused silica uncoated guard column connected to a modified packed column-type injector system

Column temperature: 200°. [NOTE—The initial temperature of the column may be adjusted so that the retention time of ethion is 3.36 relative to that of chlorpyrifos.]

Carrier gas: Helium

Flow rate: 25 mL/min. Adjust so that the retention time of chlorpyrifos is 4 min.

Makeup gas: Nitrogen, 40 mL/min

Injection volume: 5 μ L

Analysis

The following procedure is to be followed for *Chromatographic systems I and II*.

Samples: *Standard solution* and *Sample solution*

Inject the appropriate composite *Standard solution* and the *Sample solution* into the gas chromatograph, record the chromatograms, and measure the areas of all the peaks observed in the chromatograms. Compare the peak areas of any of the pesticide residues in the *Sample solution* from each chromatographic system with the peak areas that correspond to the retention times in the appropriate composite *Standard solution* from each corresponding chromatographic system.

Calculate the quantity, in ppm, of the individual specified residue found in the sample taken:

$$\text{Result} = (r_u/r_s) \times (C/W) \times 30$$

r_u = peak area of each residue from the *Sample solution*

r_s = peak area of each residue from the *Standard solution*

C = concentration of the reference pesticide in the *Standard solution* (mg/L)

W = weight of Lanolin taken (g)

Acceptance criteria

Individual specified residue: NMT 1 ppm

Total specified residues: NMT 3 ppm

SPECIFIC TESTS

• FATS AND FIXED OILS, Acid Value (Free Fatty Acids) (401)

Sample: 12.5 g

Acceptance criteria: The free acids obtained from the *Sample* require NMT 2.0 mL of 0.10 N sodium hydroxide for neutralization.

• ALKALINITY

Sample: 2.5 g

Analysis: Dissolve the *Sample* in 10 mL of ether, and add 2 drops of phenolphthalein TS.

Acceptance criteria: No red color is produced.

• WATER-SOLUBLE ACIDS AND ALKALIES

Sample: 12.5 g

Analysis: Warm the *Sample* with 50 mL of water on a steam bath, constantly stirring the mixture until the Lanolin is melted.

Acceptance criteria: The fat separates completely on cooling, leaving the water layer nearly clear and neutral to litmus. Retain the water layer for the test for *Ammonia*.

• AMMONIA

Sample solution: 10 mL of the solution from *Water-Soluble Acids and Alkalies*

Analysis: Add 1 mL of 1 N sodium hydroxide to the *Sample solution*, and boil.

Acceptance criteria: The vapors do not turn red litmus to blue.

• WATER DETERMINATION, Method I (921)

Solution A: Chloroform and methanol (3:2)

Sample solution: 250 mg/mL of Modified Lanolin in *Solution A*

Analysis: Determine the water content of a 10.0-mL portion of the *Sample solution*. Perform a blank determination on 10.0 mL of *Solution A*, and make any necessary correction.

Acceptance criteria: NMT 0.25%

• PETROLATUM

Sample: 3 g

Analysis: Heat the *Sample* on a steam bath, with frequent stirring, until it loses about 0.25% of its weight. Boil 40 mL of dehydrated alcohol with 500 mg of the dried lanolin so obtained.

Acceptance criteria: The solution is clear or NMT opalescent.

ADDITIONAL REQUIREMENTS

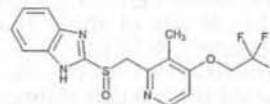
• **PACKAGING AND STORAGE:** Preserve in tight, preferably rust-proof containers, preferably at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Lanolin RS

USP Lanolin Alcohols RS

Lansoprazole



$C_{16}H_{14}F_3N_3O_2S$ 369.36

1*H*-Benzimidazole, 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-; 2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]-methyl]sulfinyl]benzimidazole [103577-45-3].

DEFINITION

Lansoprazole contains NLT 98.0% and NMT 102.0% of $C_{16}H_{14}F_3N_3O_2S$.

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

• B. ULTRAVIOLET ABSORPTION (197U)

Sample solution: 10 μ g/mL in methanol

Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile, water, and triethylamine (40:60:1). Adjust with phosphoric acid to a pH of 7.0.

Diluent: Acetonitrile, water, and triethylamine

(40:60:1). Adjust with phosphoric acid to a pH of 10.0.

System suitability solution: 0.1 mg/mL of USP Lansoprazole RS and 0.1 mg/mL of USP Lansoprazole Related Compound A RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Lansoprazole RS in *Diluent*

Sample solution: 0.1 mg/mL of Lansoprazole in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection size: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 5 between lansoprazole and lansoprazole related compound A, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lansoprazole

(C₁₆H₁₄F₃N₃O₂S) in the portion of Lansoprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lansoprazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Lansoprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Organic Impurities

• PROCEDURE

[NOTE—Store and inject the lansoprazole solutions at or below 5° using a cooled autosampler. The solutions are stable for about 24 h when stored at 5°.]

Solution A: Water

Solution B: Acetonitrile, water, and triethylamine (160:40:1). Adjust with phosphoric acid to a pH of 7.0.

Diluent: Methanol and 0.1 N sodium hydroxide (1:3)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
40	20	80
50	20	80
51	90	10
60	90	10

System suitability solution: Prepare a solution containing 25 μg/mL of USP Lansoprazole RS and 25 μg/mL of USP Lansoprazole Related Compound A RS in methanol. Transfer 1 mL of this solution into a 10-mL volumetric flask, and dilute with *Diluent* to volume.

Standard solution: Prepare a solution containing 25 μg/mL of USP Lansoprazole RS and 25 μg/mL of USP Lansoprazole Related Compound B RS in methanol. Transfer 1 mL of this solution into a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Sample solution: 2.5 mg/mL of Lansoprazole in methanol. Transfer 1 mL of this solution into a 10-mL volumetric flask, and dilute with *Diluent* to volume.

Blank: Methanol and *Diluent* (1:9)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 0.8 mL/min

Injection size: 40 μL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 6 between lansoprazole and lansoprazole related compound A

Relative standard deviation: NMT 3%

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Identify the lansoprazole peak and the peaks due to the impurities listed in *Table 2*. Measure the areas for the major peaks, excluding peaks obtained from the *Blank*.

Calculate the percentage of lansoprazole related compound B in the portion of Lansoprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for lansoprazole related compound B from the *Sample solution*

r_S = peak response for lansoprazole related compound B from the *Standard solution*

C_S = concentration of USP Lansoprazole Related Compound B RS in the *Standard solution* (μg/mL)

C_U = concentration of Lansoprazole in the *Sample solution* (μg/mL)

Calculate the percentage of lansoprazole *N*-oxide, lansoprazole sulfone, and any other individual impurity in the portion of Lansoprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response for lansoprazole from the *Standard solution*

C_S = concentration of USP Lansoprazole RS in the *Standard solution* (μg/mL)

C_U = concentration of Lansoprazole in the *Sample solution* (μg/mL)

F = relative response factor for each impurity (see *Table 2*)

Acceptance criteria

Individual impurities: See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lansoprazole <i>N</i> -oxide ^a	0.8	1.3	0.1
Lansoprazole	1.0	—	—
Lansoprazole related compound A (lansoprazole sulfone) ^b	1.1	0.82	0.4
Lansoprazole related compound B (lansoprazole sulfide) ^c	1.2	—	0.1

^a [[[(1*H*-Benzimidazole-2-yl)sulfinyl]-3-methyl-4-(2,2,2-trifluoroethoxy)-pyridine-1-oxide.

^b 2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfonyl]benzimidazole.

^c 2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-pyridin-2-yl]methyl]sulfonyl]-1*H*-benzimidazole.

Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Other individual impurity	—	1.00	0.1
Total impurities	—	—	0.6

^a [[1*H*-Benzimidazole-2-yl]sulfonyl]methyl-3-methyl-4-(2,2,2-trifluoroethoxy)-pyridine 1-oxide.

^b 2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfonyl]benzimidazole.

^c 2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-pyridin-2-yl]methyl]sulfonyl]-1*H*-benzimidazole.

SPECIFIC TESTS

• WATER DETERMINATION, Method Ia (921)

Sample: 1.0 g

[NOTE—Use 50 mL of a dehydrated mixture of pyridine and ethylene glycol (9:1 to 8:2) as the solvent.]

Acceptance criteria: NMT 0.1%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature, and protect from excessive heat.

• USP REFERENCE STANDARDS (11)

USP Lansoprazole RS

USP Lansoprazole Related Compound A RS

2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfonyl]benzimidazole.

C₁₆H₁₄F₃N₃O₃S 385.36

USP Lansoprazole Related Compound B RS

2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-pyridin-2-yl]methyl]sulfonyl]-1*H*-benzimidazole.

C₁₆H₁₄F₃N₃OS 353.36

Lansoprazole Delayed-Release Capsules

» Lansoprazole Delayed-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lansoprazole (C₁₆H₁₄F₃N₃O₂S).

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.

USP Reference standards (11)—

USP Lansoprazole RS

C₁₆H₁₄F₃N₃O₂S 369.36

USP Lansoprazole Related Compound A RS

2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfonyl]benzimidazole.

C₁₆H₁₄F₃N₃O₃S 385.36

Identification—

A: Ultraviolet Absorption (197U)—

Medium: methanol.

Procedure—Powder a portion of Capsule contents equivalent to 5 mg of lansoprazole. Add 5 mL of methanol, shake well, and centrifuge. To 0.1 mL of the supernatant, add 10 mL of methanol.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)—Proceed as directed for *Procedure for Method A* under *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*.

ACID STAGE—

Acid stage medium: 0.1 N hydrochloric acid; 500 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Procedure—Withdraw a 25-mL aliquot and then proceed immediately as directed for *Test solution* in the *Buffer stage*, leaving the remaining 475 mL in the vessel for use in the *Buffer stage*. Using a filtered portion of the aliquot, determine the amount of C₁₆H₁₄F₃N₃O₂S dissolved by employing UV absorption at the wavelength of maximum absorbance at about 306 nm, using *Acid stage medium* as the blank. Concomitantly determine the absorbance of the *Acid stage test solution* in comparison with a Standard solution of USP Lansoprazole RS having a known concentration equivalent to about 8% of the labeled amount of lansoprazole dissolved per 500 mL of *Acid stage medium*. [NOTE—A volume of methanol not to exceed 0.5% of the total volume of the Standard solution may be used to dissolve USP Lansoprazole RS prior to dilution with *Acid stage medium*.]

Tolerances—Not more than 10% of the labeled amount of C₁₆H₁₄F₃N₃O₂S is dissolved in 60 minutes.

BUFFER STAGE—

Buffer concentrate—Transfer 65.4 g of monobasic sodium phosphate, 28.2 g of sodium hydroxide, and 12 g of sodium dodecyl sulfate to a suitable container, and add enough water to dissolve. Dilute with water to 4 L, and mix well.

Blank solution—Prepare a mixture of *Acid stage medium* and *Buffer concentrate* (19:17). Adjust, if necessary, with either phosphoric acid or sodium hydroxide to a pH of 6.8.

Test solution—Add 425 mL of *Buffer concentrate* to the remaining 475 mL of solution in each vessel from the *Acid stage*. Adjust, if necessary, with either phosphoric acid or sodium hydroxide to a pH of 6.8.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Procedure—Determine the amount of C₁₆H₁₄F₃N₃O₂S dissolved in filtered portions of the *Test solution*, using the difference between the absorbances at the wavelengths of about 286 nm and 650 nm, with *Blank solution* as the blank. Concomitantly determine the absorbances of the *Test solution* in comparison with a Standard solution of USP Lansoprazole RS having a known concentration equivalent to about 70% of the labeled amount of lansoprazole dissolved in 900 mL of *Blank solution*. [NOTE—An amount of methanol not to exceed 2% of the total volume of the Standard solution may be used to dissolve USP Lansoprazole RS prior to dilution with *Blank solution*.]

Tolerances—Not less than 80% (Q) of the labeled amount of C₁₆H₁₄F₃N₃O₂S is dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

Test solution—Transfer the contents of 1 Capsule to a 100-mL volumetric flask, add 30 mL of 0.1 M sodium hydroxide, and sonicate to disintegrate. Add 65 mL of acetonitrile, cool, and dilute with acetonitrile to volume. Centrifuge a portion of the suspension and pass through a membrane filter having a 0.5-μm or finer porosity. Quantitatively dilute a volume of the filtrate with a mixture of acetonitrile and 0.1 M sodium hydroxide (7:3) to obtain a solution containing about 0.012 mg of lansoprazole per mL.

Procedure—Concomitantly determine the absorbances of the *Test solution* and a solution of USP Lansoprazole RS in the same medium and having a known concentration of about 0.012 mg of lansoprazole per mL, in 1-cm cells, at the wavelength of maximum absorbance at about 294 nm, with a suitable spectrophotometer, using a mixture of acetonitrile and 0.1 M sodium hydroxide (7:3) as the blank.

Calculate the quantity, in mg, of $C_{16}H_{14}F_3N_3O_2S$ in the Capsule taken by the formula:

$$(LC/D)(A_U / A_S)$$

in which L is the labeled quantity of lansoprazole in the Capsule; C is the concentration, in mg per mL, of USP Lansoprazole RS in the Standard solution; D is the concentration, in mg per mL, of lansoprazole in the Test solution, based on the labeled quantity of lansoprazole per Capsule and the extent of dilution; and A_U and A_S are the absorbances of the Test solution and the Standard solution, respectively.

Loss on drying (731)—Dry about 1 g of the Capsule contents in vacuum over phosphorus pentoxide at a pressure not exceeding 5 mm of mercury at 60° for 5 hours; it loses not more than 5.0% of its weight.

Assay—

Diluent, Mobile phase, and Resolution solution—Prepare as directed in the Assay under Lansoprazole.

Internal standard solution—Dissolve an accurately weighed quantity of 4'-ethoxyacetophenone in acetonitrile to obtain a solution having a known concentration of about 7.5 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Lansoprazole RS in a mixture of 0.1 M sodium hydroxide and acetonitrile (3:2) to obtain a solution having a known concentration of 3.0 mg per mL. Transfer 25.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix. Quantitatively dilute with *Diluent* to obtain a solution having a known concentration of about 0.1 mg of USP Lansoprazole RS per mL.

Assay preparation—Transfer the contents of not fewer than 10 Capsules, equivalent to about 300 mg of lansoprazole, to a 300-mL conical flask containing 60.0 mL of 0.1 M sodium hydroxide, and sonicate until completely disintegrated. Add 20.0 mL of acetonitrile and 20.0 mL of *Internal standard solution*, shake well, and centrifuge a portion of the suspension. Quantitatively dilute a volume of the supernatant with *Diluent* to obtain a solution containing about 0.1 mg of lansoprazole per mL, and pass through a membrane filter having a 0.5- μ m or finer porosity.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 285-nm detector and a 4.6-mm \times 25-cm column that contains 5- μ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*; the resolution, R , between the two major peaks is not less than 5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*; the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) in each Capsule taken by the formula:

$$(LC/D)(R_U / R_S)$$

in which L is the labeled quantity, in mg, of lansoprazole in each Capsule taken; C is the concentration, in mg per mL, of USP Lansoprazole RS in the *Standard preparation*; D is the concentration, in mg per mL, of lansoprazole in the *Assay preparation*, based on the labeled quantity of lansoprazole in the Capsules taken and the extent of dilution; and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Lansoprazole Compounded Oral Suspension

DEFINITION

Lansoprazole Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$). Prepare Lansoprazole Compounded Oral Suspension 3 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Lansoprazole delayed-release capsule(s) ^a equivalent to	300 mg
Vehicle: A mixture of Ora-Blend ^b and Sodium Bicarbonate Injection (8.4%) (1:1), a sufficient quantity to make	100 mL

^a Lansoprazole 30-mg delayed-release capsules, Dr. Reddy's Laboratory Limited, Bridgewater, NJ.

^b Perrigo Pharmaceuticals, Allegan, MI.

Empty the required number of delayed-release capsules, and pour the contents into a mortar or other suitable container. If necessary, crush the contents into a fine powder by using a pestle or other mechanical means. Wet the powder with a small amount of *Vehicle*, and triturate to make a smooth paste. Add *Vehicle* to make the mortar contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container using the remainder of *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

Alternatively, a compounded 8.4% sodium bicarbonate solution may be used instead of *Sodium Bicarbonate Injection* (8.4%). Prepare an 8.4% sodium bicarbonate solution by dissolving 8.4 g of Sodium Bicarbonate in sufficient Purified Water to make 100 mL.

ASSAY

PROCEDURE

Solution A: 10 mM sodium phosphate adjusted with sodium hydroxide to a pH of 7.5. Pass through a nylon filter of 0.45- μ m pore size, and degas.

Solution B: Acetonitrile and water (50:50)

Solution C: Water adjusted with 1 M sodium hydroxide to a pH of 6.5

Mobile phase: Acetonitrile and *Solution A* (45:55)

Standard stock solution: 3 mg/mL of USP Lansoprazole RS in *Solution B*. Mix well, and sonicate for 3 min. Store at 2°–8°.

Standard solution: Transfer 2.0 mL of the *Standard stock solution* to a 500-mL volumetric flask, and dilute with *Solution C* to volume. Centrifuge an aliquot of the solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

Sample solution: Shake each bottle of Oral Suspension thoroughly. Transfer 2.0 mL of Oral Suspension to a 500-mL volumetric flask, and dilute with *Solution C* to volume. Centrifuge an aliquot of the solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Temperatures

Column: 35°

Autosampler: 5°

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[NOTE—The retention time for lansoprazole is about 5.2 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis**Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of lansoprazole from the *Sample solution* r_S = peak response of lansoprazole from the *Standard solution* C_S = concentration of lansoprazole in the *Standard solution* (mg/mL) C_U = nominal concentration of lansoprazole in the *Sample solution* (mg/mL)

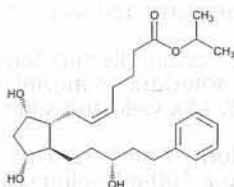
Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **PH** (791): 8.0–8.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
- **LABELING:** Label Oral Suspension to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored at 2°–8° or at controlled room temperature
- **USP REFERENCE STANDARDS** (11)
USP Lansoprazole RS

Latanoprost $C_{26}H_{40}O_5$ 432.595-Heptenoic acid, 7-[3,5-dihydroxy-2-(3-hydroxy-5-phenylpentyl)cyclopentyl]-1-methylethyl ester, [1*R*-(1*α*(*Z*),2*β*(*R**),3*α*,5*α*)]-Isopropyl (*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate [130209-82-4].**DEFINITION**Latanoprost contains NLT 94.0% and NMT 102.0% of latanoprost ($C_{26}H_{40}O_5$), calculated on the anhydrous and solvent-free basis.**[CAUTION]**—Wear protective glasses and gloves while handling the material. Avoid contact during pregnancy or while nursing.]**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197F)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY• **PROCEDURE****Mobile phase:** Chromatographic hexane and dehydrated alcohol (94:6)**System suitability solution:** Transfer 2.0 mg/mL of USP Latanoprost RS and 20 µg/mL of USP Latanoprost Related Compound A RS into a suitable volumetric flask, dissolve in dehydrated alcohol equivalent to 20% of the final volume, and dilute with chromatographic hexane to volume.**Standard solution:** Transfer 2.0 mg/mL of USP Latanoprost RS into a suitable volumetric flask, dissolve in dehydrated alcohol equivalent to 20% of the final volume, and dilute with chromatographic hexane to volume.**Sample solution:** Transfer 2.0 mg/mL of Latanoprost into a suitable volumetric flask, dissolve in dehydrated alcohol equivalent to 20% of the final volume, and dilute with chromatographic hexane to volume.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 210 nm**Column:** 4.0-mm × 25-cm; 5-µm packing L3**Column temperature:** 30°**Flow rate:** 1 mL/min**Injection volume:** 10 µL**System suitability****Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for latanoprost and latanoprost related compound A are 1.0 and 1.1, respectively.]

Suitability requirements**Resolution:** NLT 2.0 between latanoprost and latanoprost related compound A, *System suitability solution***Relative standard deviation:** NMT 1.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of latanoprost ($C_{26}H_{40}O_5$) in the portion of Latanoprost taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak area from the *Sample solution* r_S = peak area from the *Standard solution* C_S = concentration of USP Latanoprost RS in the *Standard solution* (mg/mL) C_U = concentration of Latanoprost in the *Sample solution* (mg/mL)

Acceptance criteria: 94.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.50%

• **ORGANIC IMPURITIES****Mobile phase, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.**Standard solution:** 0.04 mg/mL of USP Latanoprost RS in a mixture of chromatographic hexane and dehydrated alcohol (80:20) prepared as follows. Transfer USP Latanoprost RS into a suitable volumetric flask, dissolve in dehydrated alcohol equivalent to 20% of the final volume, and dilute with chromatographic hexane to volume.**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 2.0 between latanoprost and latanoprost related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Latanoprost taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of latanoprost from the *Standard solution*

C_S = concentration of USP Latanoprost RS in the *Standard solution* (mg/mL)

C_U = concentration of Latanoprost in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Isopropyl diphenylphosphorylpentanoate ^a	0.79	2.4	0.1
Latanoprost related compound B ^b	0.89	1.0	0.5
Latanoprost	1.00	—	—
Latanoprost related compound A ^c	1.10	1.0	3.5
Any unspecified impurity	—	1.0	0.1
Total impurities ^d	—	—	0.5

^a Isopropyl 5-(diphenylphosphoryl)pentanoate.

^b Isopropyl (Z)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*S*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate.

^c Isopropyl (E)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate.

^d Latanoprost related compound A and latanoprost related compound B are excluded.

• LIMIT OF LATANOPROST RELATED COMPOUND E

Solution A: Acetonitrile, phosphoric acid, and water (300:1:700)

Solution B: Acetonitrile, phosphoric acid, and water (800:1:200)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	0	100
15	0	100
16	100	0
21	100	0

Diluent: Acetonitrile and water (30:70)

Standard solution: 1.0 µg/mL of USP Latanoprost Related Compound E RS in *Diluent*

Sample solution: 1.0 mg/mL of Latanoprost in *Diluent*
Chromatographic system
 (See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.0-mm × 15-cm; 5-µm packing L1

Column temperature: 60°

Flow rate: 1.0 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of latanoprost related compound E in the portion of Latanoprost taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of latanoprost related compound E from the *Sample solution*

r_S = peak area of latanoprost related compound E from the *Standard solution*

C_S = concentration of USP Latanoprost Related Compound E RS in the *Standard solution* (mg/mL)

C_U = concentration of Latanoprost in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2%

SPECIFIC TESTS

• OPTICAL ROTATION, *Specific Rotation* (781S)

Sample solution: 10 mg/mL of Latanoprost in acetonitrile

Acceptance criteria: +31° to +38°

• WATER DETERMINATION, *Method 1c* (921)

Sample solution: 100 mg/mL of Latanoprost in ethyl acetate. [NOTE—Alternatively, *Water Determination* (921), *Method 1a* may be used.]

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store in a refrigerator or a freezer.

• USP REFERENCE STANDARDS (11)

USP Latanoprost RS

USP Latanoprost Related Compound A RS

Isopropyl (E)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate.

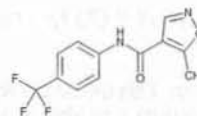
$C_{26}H_{40}O_5$ 432.59

USP Latanoprost Related Compound E RS

(Z)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoic acid.

$C_{23}H_{34}O_5$ 390.51

Leflunomide



$C_{12}H_9F_3N_2O_2$ 270.21
 4-Isioxazolecarboxamide, 5-methyl-N-[4-(trifluoromethyl)-phenyl]-;

α,α,α -Trifluoro-5-methyl-4-isoxazolecarboxy-*p*-toluidide [75706-12-6].

DEFINITION

Leflunomide contains NLT 98.0% and NMT 102.0% of $C_{12}H_9F_3N_2O_2$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
Sample: Dry the substance for 10 min at 130°.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Mobile phase: Acetonitrile, triethylamine, and water (70:1:130). Adjust with phosphoric acid to a pH of 4.
Standard solution: 0.5 mg/mL of USP Leflunomide RS in acetonitrile and *Mobile phase* (1:9). [NOTE—First dissolve in acetonitrile. Protect solutions from light.]

System suitability solution: 0.5 mg/mL of USP Leflunomide RS, 0.15 mg/mL of USP Leflunomide Related Compound B RS, and 0.05 mg/mL of USP Leflunomide Related Compound C RS in *Mobile phase*. [NOTE—Dissolve the Reference Standards in acetonitrile, and dilute with *Mobile phase*.]

Sample solution: 0.5 mg/mL of Leflunomide in acetonitrile and *Mobile phase* (1:9). [NOTE—First dissolve in acetonitrile. Protect solutions from light.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm × 12.5-cm; packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for leflunomide related compound B and leflunomide related compound C are 0.2 and 0.9, respectively.]

Suitability requirements

Resolution: NLT 1.0 between the leflunomide and leflunomide related compound C peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{12}H_9F_3N_2O_2$ in the portion of Leflunomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of Leflunomide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES**Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-Jan-2018)

Organic Impurities

- **PROCEDURE 1: LIMIT OF LEFLUNOMIDE RELATED COMPOUND A**
Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard stock solution: 0.125 mg/mL of USP Leflunomide Related Compound A RS, in acetonitrile and *Mobile phase* (1:19)

Standard solution: 0.5 µg/mL of USP Leflunomide Related Compound A RS, from the *Standard stock solution* in *Mobile phase*

Sample solution: 2.5 mg/mL of Leflunomide, in acetonitrile and *Mobile phase* (1:9)

Injection size: 20 µL

Analysis

Samples: *Standard solution* and *Sample solution*
 Calculate the percentage of leflunomide related compound A in the portion of Leflunomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of leflunomide related compound A from the *Sample solution*

r_S = peak area of leflunomide related compound A from the *Standard solution*

C_S = concentration of USP Leflunomide Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Leflunomide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.02 %

• **PROCEDURE 2**

Mobile phase, Sample solution, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.5 µg/mL of USP Leflunomide RS, from the *Standard solution* in *Mobile phase*

Sensitivity solution: 0.25 µg/mL of Leflunomide, from the *Standard solution* in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Resolution: NLT 1.0 between leflunomide and leflunomide related compound C

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Disregard any peak with an area less than the leflunomide peak from the *Sensitivity solution*. Continue the elution for two times the retention time of the leflunomide peak.]

Calculate the percentage of each related compound and any unknown impurity (see *Impurity Table 1*) in the portion of Leflunomide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area for each impurity from the *Sample solution*

r_S = peak area of leflunomide from the *Standard solution*

C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)

C_U = concentration of Leflunomide in the *Sample solution* (mg/mL)

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Methylisoxazole-carboxylic acid	0.05	1.0	0.1
Leflunomide related compound B	0.22	1.0	0.3
N-(2'-Trifluoromethyl-phenyl)-5-methylisoxazole-4-Carboxamide	0.29	1.0	0.1
2-Cyano-acetic acid-(4'-trifluoromethyl)-anilide	0.36	1.0	0.1
Leflunomide related compound C	0.94	1.0	0.1
Any other individual impurity	—	—	0.1

Impurity Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Total impurities, excluding leflunomide related compound B and leflunomide related compound C	—	—	0.2
Total impurities	—	—	0.4

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE (741):** 164°–168°
- **LOSS ON DRYING (731):** Dry a sample in a vacuum over diphosphorus pentoxide at 60° for 4 h; it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in a well-closed container. Store at a temperature not exceeding 30°.
- **USP REFERENCE STANDARDS (11)**
 - USP Leflunomide RS
 - USP Leflunomide Related Compound A RS
 - USP Leflunomide Related Compound B RS
 - USP Leflunomide Related Compound C RS

Leflunomide Tablets**DEFINITION**

Leflunomide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of leflunomide ($C_{12}H_9F_3N_2O_2$).

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION (197U)**
 - Wavelength range: 220–360 nm
 - Sample solution: 0.01 mg/mL in methanol
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Mobile phase: Acetonitrile, triethylamine, and water (70:1:130). Adjust with phosphoric acid to a pH of 4.0.

System suitability solution A: 10 µg/mL of USP Leflunomide Related Compound A RS, 1 mg/mL of USP Leflunomide Related Compound B RS, and 100 µg/mL of USP Leflunomide Related Compound C RS in a minimum amount of acetonitrile, and diluted with *Mobile phase*.

System suitability solution B: Transfer 100.0 mg of USP Leflunomide RS to a 100-mL volumetric flask. Dissolve in 2 mL of acetonitrile, add 1 mL of *System suitability solution A* and 80 mL of *Mobile phase*, and shake by mechanical means for 10 min. Dilute with *Mobile phase* to volume.

Standard solution: 1 mg/mL of USP Leflunomide RS in a minimum volume of acetonitrile, and diluted with *Mobile phase*.

Sample solution: Transfer equivalent to 100 mg of leflunomide, from finely powdered Tablets (NLT 20), to a 100-mL volumetric flask. Add 20 mL of acetonitrile, dilute with *Mobile phase* to volume, and shake by mechanical means for 10 min. Pass through a membrane filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.0-mm × 12.5-cm; packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Samples: *System suitability solution B* and *Standard solution*

[NOTE—The relative retention times for leflunomide related compound B, leflunomide related compound A, leflunomide related compound C, and leflunomide are 0.2, 0.4, 0.9, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between leflunomide related compound C and leflunomide, *System suitability solution B*

Tailing factor: NMT 3.0 for leflunomide, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of leflunomide ($C_{12}H_9F_3N_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of leflunomide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)****Test 1****Medium**

For Tablets labeled to contain 10 or 20 mg: Water, 1000 mL, deaerated

For Tablets labeled to contain 100 mg: Water containing 0.6% of polyoxyethylene (23) lauryl ether; 1000 mL, deaerated

Apparatus 2: 100 rpm

Time: 30 min

Determine the amount of leflunomide ($C_{12}H_9F_3N_2O_2$) dissolved by using one of the following methods.

Spectrometric method

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 262 nm

Standard solution: USP Leflunomide RS in *Medium*.

[NOTE—A volume of methanol not exceeding 2% of the final volume of the *Standard solution* may be used to dissolve leflunomide.]

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*, if necessary.

Chromatographic method

Mobile phase: Acetonitrile and water (1:1)

Standard solution: Transfer 22 mg of USP Leflunomide RS to a 100-mL volumetric flask. Add 40 mL of acetonitrile, and sonicate until dissolved. Add 40 mL of water, and cool to room temperature. Dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: Use portions of the solution under test passed through a suitable filter of 0.45-µm pore size.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1.5 mL/min

Injection size: 40 μL

System suitabilitySample: *Standard solution***Suitability requirements**

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the amount of leflunomide (C₁₂H₉F₃N₂O₂) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Leflunomide RS in the *Standard solution* (mg/mL) L = label claim (mg/Tablet) V = volume of *Medium*, 1000 mL**Tolerances:** NLT 80% (Q) of the labeled amount of leflunomide (C₁₂H₉F₃N₂O₂) is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution* Test 2.**Medium, Apparatus 2, Time, Spectrometric method, and Chromatographic method:** Proceed as directed for Test 1.**Tolerances:** NLT 75% (Q) of the labeled amount of leflunomide (C₁₂H₉F₃N₂O₂) is dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

Procedure for content uniformity

Mobile phase, System suitability solution A, System suitability solution B, Standard solution, Chromatographic system, and Analysis: Proceed as directed in the Assay.

Sample solution: Transfer 1 Tablet to a suitable volumetric flask, and prepare a solution having a concentration of 1 mg/mL of leflunomide. Add *Mobile phase* 50% by volume, and shake to disintegrate the Tablet. After the Tablet is completely disintegrated, add acetonitrile 20% by volume, dilute with *Mobile phase* to volume, and shake again. Pass through a membrane filter.**IMPURITIES**

- PROCEDURE**

Mobile phase, System suitability solution A, System suitability solution B, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

AnalysisSamples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 r_U = peak response of each individual impurity from the *Sample solution* r_T = sum of all the peak responses of the related compounds and leflunomide from the *Sample solution***Acceptance criteria**

Leflunomide related compound A: NMT 0.1%

Leflunomide related compound B: NMT 3.5%

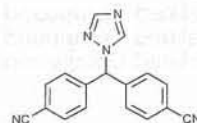
Leflunomide related compound C: NMT 0.2%

Individual impurities: NMT 0.2%

Total impurities: NMT 4.0%

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant, and humidity-resistant containers.
- LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if Test 1 is not used.
- USP REFERENCE STANDARDS (11)**
 - USP Leflunomide RS
 - USP Leflunomide Related Compound A RS
 - USP Leflunomide Related Compound B RS
 - USP Leflunomide Related Compound C RS

LetrozoleC₁₇H₁₁N₅

285.30

Benzonitrile, 4,4'-(1H-1,2,4-triazol-1-ylmethylene)bis-; 4,4'-(1H-1,2,4-Triazol-1-ylmethylene)dibenzonitrile [112809-51-5].

DEFINITIONLetrozole contains NLT 98.0% and NMT 102.0% of C₁₇H₁₁N₅, calculated on the anhydrous basis.**IDENTIFICATION**

- A. INFRARED ABSORPTION (197M)**
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

- PROCEDURE**

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	30	70

Diluent: Acetonitrile and water (3:7)**Standard solution:** 10 μg/mL of USP Letrozole RS in *Diluent*. [NOTE—Dissolve USP Letrozole RS in acetonitrile, then dilute with water.]**Sample solution:** 10 μg/mL of Letrozole in *Diluent*. [NOTE—Dissolve Letrozole in acetonitrile, then dilute with water.]

Chromatographic system(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 12.5-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection size: 20 μL

System suitabilitySample: *Standard solution***Suitability requirements**

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of C₁₇H₁₁N₅ in the portion of Letrozole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*r_S = peak response from the *Standard solution*C_S = concentration of USP Letrozole RS in the *Standard solution* (mg/mL)C_U = nominal concentration of letrozole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES**Inorganic Impurities**

- **RESIDUE ON IGNITION** <281>: NMT 0.1%

Delete the following:

- **HEAVY METALS**, *Method II* <231>: 10 ppm (Official 1-Jan-2018)

Organic Impurities**• PROCEDURE**Solution A, Solution B, Mobile phase, Chromatographic system, and Diluent: Proceed as directed in the *Assay*.System suitability solution: 2 μg/mL of USP Letrozole Related Compound A RS and 10 μg/mL of USP Letrozole RS in *Diluent*. [NOTE—Dissolve Letrozole and USP Letrozole Related Compound A RS in acetonitrile, then dilute with water.]Standard solution: 1 μg/mL of USP Letrozole RS in *Diluent*. [NOTE—Dissolve USP Letrozole RS in acetonitrile, then dilute with water.]

Sample solution: Transfer 25 mg of Letrozole to a 250-mL volumetric flask. Dissolve in 75 mL of acetonitrile, and dilute with water to volume.

System suitabilitySamples: *System suitability solution* and *Standard solution***Suitability requirements**Resolution: NLT 2.0 between letrozole related compound A and letrozole, *System suitability solution*Relative standard deviation: NMT 10.0%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Letrozole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*r_S = peak response of letrozole from the *Standard solution*C_S = concentration of USP Letrozole RS in the *Standard solution* (mg/mL)C_U = concentration of Letrozole in the *Sample solution* (mg/mL)**Acceptance criteria**Individual impurities: See *Impurity Table 1*.

Total unspecified impurities: NMT 0.3%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Letrozole related compound A ^a	0.67	0.3
Letrozole	1.0	—
4,4',4''-Methanetriyl-tribenzonitrile	2.4	0.2
Any unspecified impurity	—	0.1

^a 4,4'-(1*H*-1,3,4-triazol-1-ylmethylene)dibenzonitrile.

[NOTE—Disregard any impurity peaks less than 0.05%.]

SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* <921>: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers at controlled room temperature.

- **USP REFERENCE STANDARDS** <11>

USP Letrozole RS

USP Letrozole Related Compound A RS

4,4'-(1*H*-1,3,4-Triazol-1-ylmethylene)dibenzonitrile.C₁₇H₁₁N₅ 285.31**Letrozole Tablets****DEFINITION**Letrozole Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of letrozole (C₁₇H₁₁N₅).**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** <201>

Sample solution: Equivalent to 2 mg/mL of letrozole from powdered Tablets in methanol. [NOTE—Shake thoroughly, sonicate for 10 min, and centrifuge.]

Application volume: 5 μL

Developing solvent system: Ethyl acetate and methanol (9:1)

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Mobile phase: Acetonitrile and water (48:52)

Diluent: Acetonitrile and water (30:70)

Standard stock solution: 0.2 mg/mL of USP Letrozole RS in *Diluent*. [NOTE—Dissolve letrozole in acetonitrile, and then dilute with water.]Standard solution: 10 μg/mL of USP Letrozole RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: Equivalent to 50 mg of letrozole from Tablets in a 250-mL volumetric flask. Add 20 mL of water and shake for 5 min to dissolve the Tablets. Add 75 mL of acetonitrile, shake for 30 min, and dilute with water to volume. Centrifuge a portion of the solution.

Sample solution: 10 μg/mL of letrozole in *Mobile phase* from the *Sample stock solution*

Chromatographic system(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 12.5-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of letrozole (C₁₇H₁₁N₅) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Letrozole RS in the *Standard solution* (μg/mL) C_U = nominal concentration of letrozole in the *Sample solution* (μg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS**• DISSOLUTION <711>****Test 1**

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 100 rpm

Time: 30 min

Standard solution: Transfer USP Letrozole RS to a suitable volumetric flask, dissolve in acetonitrile equivalent to 10% of the final volume, and dilute with *Medium* to volume to obtain a solution of 0.05 mg/mL of letrozole. Dilute this solution with *Medium* to obtain a solution of 0.005 mg/mL of letrozole.

Sample solution: Centrifuge a portion of the solution under test at 4000 rpm for 5 min.

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*, except use an injection volume of 200 μL.**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of letrozole (C₁₇H₁₁N₅) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Letrozole RS in the *Standard solution* (mg/mL) L = label claim (mg/Tablet) V = volume of *Medium*, 500 mLTolerances: NLT 80% (Q) of the labeled amount of letrozole (C₁₇H₁₁N₅) is dissolved.**Test 2**

Medium: 0.1 N hydrochloric acid solution adjusted with 50% sodium hydroxide (NaOH) to a pH of 1.2; 900 mL, deaerated

Apparatus 2: 75 rpm

Time: 30 min

Mobile phase: Acetonitrile and water (45:55)

Standard stock solution: 0.3 mg/mL of USP Letrozole RS in *Mobile phase*Standard solution: 3.0 μg/mL of USP Letrozole RS in *Medium* from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter of 35-μm pore size.

Chromatographic system(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 100 μL

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of letrozole (C₁₇H₁₁N₅) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Letrozole RS in the *Standard solution* (mg/mL) L = label claim (mg/Tablet) V = volume of *Medium*, 900 mLTolerances: NLT 80% (Q) of the labeled amount of letrozole (C₁₇H₁₁N₅) is dissolved.**Test 3**

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 75 rpm

Time: 30 min

Mobile phase: Acetonitrile and water (48:52)

Standard stock solution: 0.25 mg/mL of USP Letrozole RS in *Mobile phase*Standard solution: 0.005 mg/mL of USP Letrozole RS in *Medium* from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size and discard the first few mL of the filtrate.

Chromatographic system(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 50 μL

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of letrozole (C₁₇H₁₁N₅) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Letrozole RS in the *Standard solution* (mg/mL) L = label claim (mg/Tablet) V = volume of *Medium*, 500 mLTolerances: NLT 80% (Q) of the labeled amount of letrozole (C₁₇H₁₁N₅) is dissolved.**• UNIFORMITY OF DOSAGE UNITS <905>: Meet the requirements****IMPURITIES****• ORGANIC IMPURITIES**

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
25	30	70

Diluent: Prepare as directed in the Assay.

System suitability solution: 10 µg/mL of USP Letrozole RS and 2 µg/mL of USP Letrozole Related Compound A RS in *Diluent*. [NOTE—Dissolve letrozole and letrozole related compound A in acetonitrile, then dilute with water.]

Standard solution: 1 µg/mL of USP Letrozole RS in *Diluent*. [NOTE—Dissolve letrozole in acetonitrile, then dilute with water.]

Sample solution: Nominally 0.1 mg/mL of letrozole in *Diluent*. Shake the whole Tablets (NLT 10) for about 15 min in a portion of *Diluent* to aid in dissolution. Centrifuge, and use the supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 12.5-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between letrozole and letrozole related compound A, *System suitability solution*

Relative standard deviation: NMT 10.0% for letrozole, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of letrozole from the *Standard solution*

C_S = concentration of USP Letrozole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of letrozole in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Letrozole related compound A ^a	0.67	—
Letrozole	1.0	—
4,4',4''-Methanetriyltribenzonitrile	2.4	—
Any unspecified impurity	—	0.1
Total unspecified impurities	—	0.3

^a 4,4'-(1*H*-1,3,4-Triazol-1-ylmethylene)dibenzonitrile.

[NOTE—Letrozole related compound A and 4,4',4''-Methanetriyltribenzonitrile are process impurities and are controlled in the drug substance monograph.]

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• USP REFERENCE STANDARDS (11)

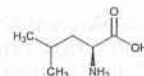
USP Letrozole RS

USP Letrozole Related Compound A RS

4,4'-(1*H*-1,3,4-Triazol-1-ylmethylene)dibenzonitrile.

C₁₇H₁₁N₅ 285.31

Leucine



C₆H₁₃NO₂

L-Leucine [61-90-5].

131.17

DEFINITION

Leucine contains NLT 98.5% and NMT 101.5% of L-leucine (C₆H₁₃NO₂), calculated on the dried basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

ASSAY

• PROCEDURE

Sample: 130 mg of Leucine

Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with the *Titrant*. Perform the blank determination.

Calculate the percentage of leucine (C₆H₁₃NO₂) in the portion of the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 131.2 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.4%

• **CHLORIDE AND SULFATE, Chloride** (221)

Standard solution: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.73 g of Leucine

Acceptance criteria: NMT 0.05%

• **CHLORIDE AND SULFATE, Sulfate** (221)

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.33 g of Leucine

Acceptance criteria: NMT 0.03%

• **IRON** (241): NMT 30 ppm

Delete the following:

• **HEAVY METALS, Method II** (231): NMT 15 ppm • (Official 1-

Jan-2018)

• RELATED COMPOUNDS

Buffer solution: 0.2 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.8.

Mobile phase: Acetonitrile and *Buffer solution* (2:98)

System suitability solution: 0.25 mg/mL each of USP L-Leucine RS and USP L-Isoleucine RS in *Mobile phase*

Standard solution: 0.025 mg/mL of USP L-Isoleucine RS in *Mobile phase*

Sample solution: 5.0 mg/mL of Leucine in *Mobile phase*

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 3-μm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for isoleucine and leucine are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between leucine and isoleucine

Relative standard deviation: NMT 2.0% each for leucine and isoleucine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of isoleucine in the portion of Leucine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of isoleucine from the *Sample solution*

r_S = peak response of isoleucine from the *Standard solution*

C_S = concentration of USP L-Isoleucine RS in the *Standard solution* (mg/mL)

C_U = concentration of Leucine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Leucine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_T = sum of the peak responses of all the peaks from the *Sample solution*

Acceptance criteria

Isoleucine: NMT 0.8%

Any unspecified impurity: NMT 0.2%

Total unspecified impurities: NMT 1.0%

SPECIFIC TESTS

• OPTICAL ROTATION, *Specific Rotation* (781S)

Sample solution: 40 mg/mL in 6 N hydrochloric acid

Acceptance criteria: +14.9° to +17.3°

• pH (791)

Sample solution: 10 mg/mL in water

Acceptance criteria: 5.5–7.0

• LOSS ON DRYING (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

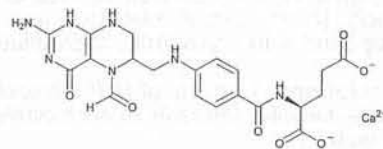
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• USP REFERENCE STANDARDS <11>

USP L-Isoleucine RS

USP L-Leucine RS

Leucovorin Calcium



$C_{20}H_{21}CaN_7O_7$

511.50

L-Glutamic acid, N-[4-[[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridiny]methyl]amino]benzoyl]-, calcium salt (1:1);

Calcium N-[p-[[[(6R)-2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridiny]methyl]amino]benzoyl]-L-glutamate (1:1) [1492-18-8].

DEFINITION

Leucovorin Calcium contains NLT 95.0% and NMT 105.0% of leucovorin calcium ($C_{20}H_{21}CaN_7O_7$), calculated on the anhydrous basis.

IDENTIFICATION

• A. INFRARED ABSORPTION <197K>

Do not dry specimens.

Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Use only freshly deionized water wherever water is specified throughout this *Procedure*. Use low-actinic glassware for solutions containing leucovorin calcium, and otherwise protect the solutions from unnecessary exposure to light. Complete the assay without prolonged interruption.

Solution A: 250 mg/mL of tetrabutylammonium hydroxide in methanol

Solution B: 276 mg/mL of monobasic sodium phosphate monohydrate in water

Mobile phase: Mix 15 mL of *Solution A* with 835 mL of water. Add 125 mL of acetonitrile, adjust with *Solution B* to an apparent pH of 7.5 ± 0.1 , dilute with water to 1000 mL, and filter. Adjust the concentration of acetonitrile, if necessary.

Diluent: Mix 15 mL of *Solution A* with 900 mL of water, and adjust with *Solution B* to a pH of 7.5 ± 0.1 . Dilute with water to 1000 mL.

Standard solution: 0.175 mg/mL of USP Leucovorin Calcium RS in *Diluent*

Sample solution: 0.2 mg/mL of Leucovorin Calcium in *Diluent*

System suitability stock solution: 0.175 mg/mL of folic acid in *Diluent*

System suitability solution: *System suitability stock solution* and *Standard solution* (1:4)

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 1–2 mL/min

Injection volume: 15 μL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for leucovorin and folic acid are 1.0 and about 1.6, respectively.]

Suitability requirements

Resolution: NLT 3.6 between leucovorin calcium and folic acid

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of leucovorin calcium ($C_{20}H_{21}CaN_7O_7$) in the portion of Leucovorin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Leucovorin Calcium RS in the *Standard solution* (mg/mL)

C_U = concentration of Leucovorin Calcium in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% on the anhydrous basis

IMPURITIES**Delete the following:**

- **HEAVY METALS, Method II (231):** 50 ppm (Official 1-Jan-2018)

SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 17.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**
USP Leucovorin Calcium RS

Leucovorin Calcium Injection**DEFINITION**

Leucovorin Calcium Injection is a sterile solution of leucovorin calcium ($C_{20}H_{21}CaN_7O_7$) in Water for Injection. It contains NLT 90.0% and NMT 120.0% of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$).

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

Analysis: Transfer a volume of Injection, equivalent to 6 mg of leucovorin calcium, to a glass-stoppered, 50-mL centrifuge tube. Add 40 mL of acetone, mix, centrifuge for a few min, and decant and discard the liquid phase. Repeat the washing process with an additional 40 mL of acetone. Dry the precipitate so obtained with a stream of dry nitrogen.

Acceptance criteria: Meets the requirements

ASSAY• **PROCEDURE**

Use only freshly deionized water wherever water is specified throughout this *Procedure*. Use low-actinic glassware for solutions containing leucovorin calcium, and otherwise protect the solutions from unnecessary exposure to light. Complete the assay without prolonged interruption.

Solution A: 250 mg/mL of tetrabutylammonium hydroxide in methanol

Solution B: 276 mg/mL of monobasic sodium phosphate monohydrate in water

Mobile phase: Mix 15 mL of *Solution A* with 835 mL of water. Add 125 mL of acetonitrile, adjust with *Solution B* to an apparent pH of 7.5 ± 0.1 , dilute with water to 1000 mL, and filter. Adjust the concentration of acetonitrile, if necessary.

Diluent: Mix 15 mL of *Solution A* with 900 mL of water, and adjust with *Solution B* to a pH of 7.5 ± 0.1 . Dilute with water to 1000 mL.

Standard solution: 0.175 mg/mL of USP Leucovorin Calcium RS in *Diluent*

Sample solution: Transfer a measured volume of Injection, equivalent to 9 mg of leucovorin, to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pipet 25 mL of this solution into a 60-mL separator, add 25 mL of methylene chloride, shake the mixture, allow the layers to separate, and discard the methylene chloride extract. Repeat the extraction with two more 25-mL portions of methylene chloride, discarding the methylene chloride extracts. Filter the aqueous layer, discarding the first 5 mL of the filtrate, and collect the remaining filtrate in a glass-stoppered conical flask.

System suitability stock solution: 0.175 mg/mL of folic acid in *Diluent*

System suitability solution: *System suitability stock solution* and *Standard solution* (1:4)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm \times 30-cm; packing L1

Flow rate: 1–2 mL/min

Injection volume: 15 μ L

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for leucovorin and folic acid are 1.0 and about 1.6, respectively.]

Suitability requirements

Resolution: NLT 3.6 between leucovorin calcium and folic acid

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Leucovorin Calcium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of leucovorin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of leucovorin, 473.45

M_{r2} = molecular weight of leucovorin calcium, 511.50

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

- **pH (791):** 6.5–8.5

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 1.95 USP Endotoxin Units/mg of leucovorin calcium

- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose, light-resistant containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS (11)**

- USP Endotoxin RS
- USP Leucovorin Calcium RS

Leucovorin Calcium Tablets

DEFINITION

Leucovorin Calcium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$).

IDENTIFICATION

- A.** **Sample:** Equivalent to 200 mg of leucovorin calcium from finely powdered Tablets
Analysis: Transfer the *Sample* to a conical flask. Add 10 mL of water, shake vigorously, sonicate for 10 min, and filter. Transfer the filtrate to a stoppered centrifuge tube, add 125 mg of ammonium oxalate, shake vigorously, and centrifuge until a clear supernatant is obtained. Transfer the supernatant to another stoppered centrifuge tube, add 1 mL of methanol and 3 drops of hydrochloric acid, and shake vigorously. If the preparation is cloudy, add methanol until a clear solution is obtained, and filter if necessary to remove any undissolved material. Cool the preparation at 0° until a precipitate forms, and centrifuge for 1–2 min. [NOTE—The cooling and centrifuging steps may be repeated if necessary to increase the amount of precipitate collected.] Decant the supernatant, add 2 mL of methanol to the tube, shake vigorously to dissolve the precipitate, and transfer the contents to a beaker. Evaporate under a current of air to dryness, and dry the residue at 50° for 30 min.
Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue exhibits maxima only at the same wavelengths as that of a similar preparation of USP Leucovorin Calcium RS.
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Diluent: Methanol and water (20:80)

Mobile phase: 5 mM tetrabutylammonium phosphate in *Diluent*. Adjust with 50% (w/v) sodium hydroxide to a pH of 7.5.

Standard solution: 0.5 mg/mL of USP Leucovorin Calcium RS and 10 µg/mL of USP 10-Formylfolic Acid RS in water

Sample solution: Transfer finely powdered Tablets (NLT 20), equivalent to 50 mg of leucovorin, to a 100-mL volumetric flask. Add 50 mL of water, sonicate for 30 min, dilute with water to volume, mix, and filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 2.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for leucovorin and 10-formylfolic acid are about 1.0 and 2.3, respectively.]

Suitability requirements

Resolution: NLT 1.5 between leucovorin and 10-formylfolic acid

Relative standard deviation: NMT 2.0% for leucovorin

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak area of the *Sample solution*
- r_S = peak area of the *Standard solution*
- C_S = concentration of USP Leucovorin Calcium RS in the *Standard solution* (mg/mL)
- C_U = nominal concentration of leucovorin in the *Sample solution* (mg/mL)
- M_{r1} = molecular weight of leucovorin, 473.45
- M_{r2} = molecular weight of leucovorin calcium, 511.50

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Detector: UV, at a maximum of about 284 nm

Standard solution: USP Leucovorin Calcium RS in *Medium*

Sample solution: Use filtered portion of solution under test, and dilute with water if necessary to a concentration similar to that of the *Standard solution*.

Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

- A_U = absorbance of the *Sample solution*
- A_S = absorbance of the *Standard solution*
- C_S = concentration of the *Standard solution* (mg/mL)
- V = volume of *Medium*, 900 mL
- D = dilution factor
- M_{r1} = molecular weight of leucovorin, 473.45
- M_{r2} = molecular weight of leucovorin calcium, 511.50
- L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905)

Analysis for content uniformity

Standard solution: 10 µg/mL of USP Leucovorin Calcium RS

Sample solution: 10 µg/mL of leucovorin calcium, use individual intact Tablets.

Blank: Water

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Cell: 1 cm

Analytical wavelength: UV, at maxima about 284 nm

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) in each Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- A_U = absorbance of the *Sample solution*
- A_S = absorbance of the *Standard solution*
- C_S = concentration of USP Leucovorin Calcium RS in the *Standard solution* (µg/mL)
- C_U = nominal concentration of leucovorin in the *Sample solution* (µg/mL)
- M_{r1} = molecular weight of leucovorin, 473.45
- M_{r2} = molecular weight of leucovorin calcium, 511.50

Acceptance criteria: Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Diluent, Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each impurity peak

r_T = sum of the responses of all the peaks

Acceptance criteria

Individual impurities: NMT 2.5%

Total impurities: NMT 4.0 %

ADDITIONAL REQUIREMENTS

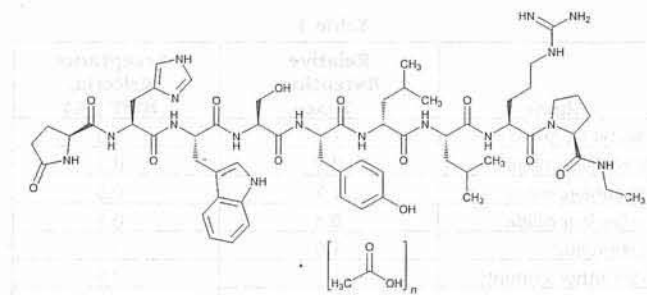
• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light, at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**

USP 10-Formylfolic Acid RS

USP Leucovorin Calcium RS

Leuprolide Acetate



$C_{59}H_{84}N_{16}O_{12} \cdot (C_2H_4O_2)_n$, $n = 1$ or 2 1209.41 (as free base)
Luteinizing hormone-releasing factor, 6-D-leucine-9-(N-ethyl-L-prolinamide)-10-deglycinamide acetate (salt);
5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide acetate (salt)
[74381-53-6].

DEFINITION

Leuprolide Acetate is a synthetic nonapeptide agonist analog of luteinizing hormone-releasing factor. It contains NLT 97.0% and NMT 103.0% of leuprolide ($C_{59}H_{84}N_{16}O_{12}$), calculated on the anhydrous, acetic acid-free basis.

[NOTE—Due to the hygroscopic nature of this material, analyses are performed immediately after opening the container in a glove box under dry nitrogen purge.]

[CAUTION—Leuprolide Acetate is a potent hormonal manipulator. Avoid skin contact and inhalation of dusts and mists.]

IDENTIFICATION

• **A. INFRARED ABSORPTION (197K)**

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: 15.2 mg/mL of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile and *n*-propyl alcohol (3:2)

Mobile phase: *Solution A* and *Solution B* (17:3)

Standard stock solution: 1 mg/mL of USP Leuprolide Acetate RS in *Mobile phase*

Standard solution: 50 µg/mL. Dilute 5.0 mL of the *Standard stock solution* with *Mobile phase* to 100.0 mL.

Degradation standard solution: Dilute 5.0 mL of the *Standard stock solution* with water to 50.0 mL. Transfer 5 mL of the solution to a scintillation vial. Add 100 µL of 1 N sodium hydroxide solution, cap tightly, and shake vigorously. Place in an oven at 100° for 60 min. Remove, allow to cool, add 50 µL of 1 M phosphoric acid, recap, and shake vigorously to mix.

Sample solution: 50 µg/mL of Leuprolide Acetate in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: 1–1.5 mL/min

Injection size: 20 µL

System suitability

Samples: *Mobile phase*, *Standard solution*, and *Degradation standard solution*

[NOTE—Chromatograph the *Mobile phase*, and verify that no extraneous peaks are present.]

[NOTE—The relative retention times for the degradation product and leuprolide are about 0.90 and 1.0, respectively.]

Suitability requirements

Retention time: 41–49 min for leuprolide, *Degradation standard solution*

Resolution: NLT 1.5 between leuprolide and the degradation product, *Degradation standard solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 1.5% for leuprolide acetate, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of leuprolide ($C_{59}H_{84}N_{16}O_{12}$) in the portion of Leuprolide Acetate taken:

$$\text{Result} = [(r_U/r_S) \times (C_S/C_U) \times P \times M \times 100]/(100 - AC - WC)$$

r_U = peak area of the *Sample solution*

r_S = peak area of the *Standard solution*

C_S = concentration of USP Leuprolide Acetate RS in the *Standard solution* (µg/mL)

C_U = concentration of Leuprolide Acetate in the *Sample solution* (µg/mL)

P = designated purity of USP Leuprolide Acetate RS (%)

M = $(100 - H)/100$, where H is equal to the water content of USP Leuprolide Acetate RS

AC = acetic acid content (%)

WC = water content (%)

Acceptance criteria: 97.0%–103.0% on the anhydrous and acetic acid-free basis

OTHER COMPONENTS

• CONTENT OF ACETIC ACID

Diluent: Methanol, adjusted with phosphoric acid to a pH of 2.5

Standard solution: Pipet 2.0 mL of glacial acetic acid into a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Transfer 4.0 mL of the solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

to obtain a solution having a known concentration of about 0.08 mg/mL.

Sample solution: Transfer about 100 mg of Leuprolide Acetate, accurately weighed, to a 100-mL volumetric flask, and dissolve in and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m fused-silica capillary column that contains a 1.2-μm film of phase G35

Temperature

Column: 100°

Injection port: 200°

Detector: 250°

Carrier gas: Helium

Flow rate: 10 mL/min

Injection size: 1.0 μL

Injection type: Splitless mode

System suitability

Samples: *Diluent* and *Standard solution*

Suitability requirements

Blank: Chromatograph the *Diluent*, and verify that there are no interfering peaks.

Column efficiency: NLT 15,000 theoretical plates, *Standard solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 2.0% for glacial acetic acid, for replicate injections of the *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acetic acid (C₂H₄O₂) in the portion of Leuprolide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (839.2/W_U)$$

r_U = peak area of the *Sample solution*

r_S = peak area of the *Standard solution*

W_U = weight of Leuprolide Acetate taken to prepare the *Sample solution* (mg)

Acceptance criteria: 4.7%–9.0%

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.3%

• CHROMATOGRAPHIC PURITY

Solution A: 15.2 mg/mL of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0 before final dilution.

Solution B: Acetonitrile and *n*-propyl alcohol (3:2)

Mobile phase: *Solution A* and *Solution B* (17:3)

Standard stock solution: 1 mg/mL of USP Leuprolide Acetate RS in *Mobile phase*

Standard solution: Dilute 1.0 mL of the *Standard stock solution* with *Mobile phase* to 100.0 mL.

Degradation standard solution: Dilute 5 mL of *Standard stock solution* with water to 50.0 mL. Transfer 5 mL of the solution to a scintillation vial. Add 100 μL of 1 N sodium hydroxide solution, tightly cap, and shake vigorously. Place in an oven at 100° for 60 min. Remove, allow to cool, add 50 μL of 1 M phosphoric acid, recap, and shake vigorously to mix.

Sample solution: Transfer about 100 mg of Leuprolide Acetate to a 100-mL volumetric flask, and dissolve in and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 3-μm packing L1

Flow rate: 1–1.5 mL/min

Injection size: 20 μL

System suitability

Samples: *Mobile phase*, *Standard solution*, *Degradation standard solution*, and *Sample solution*

[NOTE—Chromatograph the *Mobile phase*, and verify that no extraneous peaks are present.]

Suitability requirements

Retention time: 41–49 min for leuprolide, *Degradation standard solution*

Resolution: NLT 1.5 between leuprolide and the degradation product, *Degradation standard solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 1.5% for leuprolide acetate, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Record the chromatograms for 90 min.]

Calculate the percentage of each impurity in the portion of leuprolide acetate [C₅₉H₈₄N₁₆O₁₂ · (C₂H₄O₂)_n] taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times M$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response of leuprolide from the *Standard solution*

C_S = concentration of USP Leuprolide Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Leuprolide Acetate in the *Sample solution* (mg/mL)

P = designated purity of USP Leuprolide Acetate RS (%)

M = (100 – H)/100, where H is equal to the water content of USP Leuprolide Acetate RS

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acetyl-leuprolide	1.5	1.0
D-His-leuprolide	0.9	0.5
L-Leu ⁶ -leuprolide	1.2	0.5
D-Ser-leuprolide	0.8	0.5
Leuprolide	1.0	—
Any other impurity	—	0.5
Total impurities	—	2.5

SPECIFIC TESTS

• AMINO ACID CONTENT

[NOTE—Use a suitable, validated procedure (see *Biotechnology-Derived Articles—Amino Acid Analysis* (1052)).]

Standard solutions: Prepare a solution having known equimolar amounts of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine with half the equimolar amount of L-cystine. For the validation of the method, an appropriate internal standard, such as norleucine, is used. Prepare a separate, equimolar solution of L-tryptophan.

Sample solution: Transfer 64 mg of Leuprolide Acetate to a suitable vessel. Dissolve in 1.0 mL of water. Transfer 0.10 mL of this solution to a vacuum hydrolysis tube. Add 2.0 mL of 6 N hydrochloric acid, evacuate the tube, and heat for 16 h at 120°. Transfer 0.10 mL of the hydrolysate so obtained to a suitable vessel, add 1 mL of water, and lyophilize. Dissolve in and dilute to a suitable volume in a buffer solution suitable for amino acid analysis.

Analysis: Inject equal volumes of the *Standard solution* and *Sample solution* into the amino acid analyzer, and record and measure the responses for each amino acid peak. Express the content of each amino acid in moles.

Calculate the relative proportions of the amino acids in the *Sample solution*, taking one-seventh of the sum of the number of moles of histidine, glutamic acid, leucine, proline, tyrosine, and arginine as equal to one.

Acceptance criteria: 0.85–1.1 moles each of glutamic acid, proline, tyrosine, histidine, and arginine per mole of Leuprolide Acetate; 1.8–2.2 moles of leucine per mole of Leuprolide Acetate; serine and tryptophan are also present.

• **OPTICAL ROTATION, Specific Rotation (781S)**

Sample solution: 10 mg/mL, in 1% acetic acid

Acceptance criteria: -38.0° to -42.0° expressed on an anhydrous, acetic acid-free basis

• **WATER DETERMINATION, Method 1c (921):** NMT 8.0%

• **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 166.7 USP Endotoxin Units/mg of leuprolide acetate.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Store at a temperature not higher than 30° .

• **USP REFERENCE STANDARDS (11)**

USP Endotoxin RS

USP Leuprolide Acetate RS

Levalbuterol Inhalation Solution

DEFINITION

Levalbuterol Inhalation Solution is a sterile, aqueous solution of Levalbuterol Hydrochloride, prepared with Sodium Chloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of levalbuterol ($C_{13}H_{21}NO_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Phosphoric acid in water (1 in 1000)

Solution B: Acetonitrile, methanol, water, and phosphoric acid (350:350:300:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	91.5	8.5
15	91.5	8.5
15.01	0	100
20	0	100
20.01	91.5	8.5
30	91.5	8.5

Diluent: Dissolve 9.0 g of sodium chloride in 950 mL of water. Adjust with dilute sulfuric acid to a pH of 4.0, and dilute with water to 1000 mL. Mix, and pass through a filter of 0.45- μ m pore size.

Standard solution: 0.1 mg/mL of USP Levalbuterol Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.1 mg/mL of levalbuterol hydrochloride (equivalent to 0.087 mg/mL of levalbuterol free base) in *Diluent* from an appropriately diluted volume of Inhalation Solution

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 5500 theoretical plates

Tailing factor: NMT 2.3

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levalbuterol ($C_{13}H_{21}NO_3$) in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of levalbuterol hydrochloride from the *Sample solution*

r_S = peak response of levalbuterol hydrochloride from the *Standard solution*

C_S = concentration of USP Levalbuterol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levalbuterol in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of levalbuterol (free base), 239.31

M_{r2} = molecular weight of levalbuterol hydrochloride, 275.77

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Diluent, and Sample solution: Prepare as directed in the *Assay*.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	70	30
50	28	72
50.01	0	100
55	0	100
55.01	100	0
70	100	0

System suitability solution: Prepare a solution containing the following in *Diluent*.

USP Levalbuterol Hydrochloride RS, 100 μ g/mL

USP Levalbuterol Related Compound A RS, 0.05 μ g/mL

USP Levalbuterol Related Compound B RS, 0.05 μ g/mL

USP Levalbuterol Related Compound C RS, 0.05 μ g/mL

USP Levalbuterol Related Compound D RS, 0.05 μ g/mL

USP Levalbuterol Related Compound E RS, 0.05 µg/mL
USP Levalbuterol Related Compound F RS, 0.05 µg/mL

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 4.9 between levalbuterol and levalbuterol related compound A; NLT 1.5 between levalbuterol related compound B and levalbuterol related compound C

Tailing factor: NMT 4.0 for the levalbuterol peak

Analysis

Sample: *Sample solution*

[NOTE—Integrate all peaks with an area greater than 0.05% of the area corresponding to the levalbuterol peak.]

Calculate the percentage of each impurity in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the responses of all the peaks

F = relative response factor for each impurity (see Table 3)

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Hydroxyalbuterol ^a	0.90	1.0	0.10
Levalbuterol	—	—	—
Levalbuterol related compound A ^b	1.2	—	—
Levalbuterol related compound H ^{b,c}	1.3	—	—
Levalbuterol related compound B ^b	1.5	—	—
Levalbuterol related compound C ^b	1.6	—	—
Levalbuterol related compound D	1.7	3.0	0.08
Levalbuterol related compound E ^b	2.1	—	—
Levalbuterol related compound F ^b	3.5	—	—
Any individual unspecified degradation product	—	—	0.10
Total impurities	—	—	0.70

^a 5-[2-(*tert*-Butylamino)-1-hydroxyethyl]-3-(hydroxymethyl)benzene-1,2-diol.

^b Process impurity, included for identification purposes only. Not to be included in the Total impurities.

^c 4-[2-(*tert*-Butylamino)-1-methoxyethyl]-2-(hydroxymethyl)phenol.

• ENANTIOMERIC PURITY

Mobile phase: Acetonitrile, methanol, acetic acid, and triethylamine (500:500:3:1)

Diluent: *Mobile phase*

System suitability solution: 0.10 mg/mL of USP Levalbuterol Hydrochloride RS and 0.04 mg/mL of USP Albuterol RS in *Diluent*

Sample solution: Inhalation Solution

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; 5-µm packing L63

Flow rate: 1 mL/min

Injection volume: 10 µL of the *System suitability solution* and a suitable volume of *Sample solution* to obtain 4.2 µg of levalbuterol injected on the column

Run time: NLT 30 min

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times of levalbuterol and (S)-albuterol are 1.0 and 1.16, respectively.]

Suitability requirements

Resolution: NLT 3.0 between levalbuterol and (S)-albuterol

Tailing factor: NMT 1.6 for levalbuterol and NMT 2.0 for (S)-albuterol

Relative standard deviation: NMT 20% for (S)-albuterol, for three injections

Analysis

Sample: *Sample solution*

Calculate the percentage of (S)-albuterol in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of (S)-albuterol

r_T = sum of the peak responses of levalbuterol and (S)-albuterol

Acceptance criteria: NMT 2.50% of (S)-albuterol in the *Sample solution*

SPECIFIC TESTS

• **STERILITY TESTS** <71>: Meets the requirements

• **pH** <791>: 3.3–4.5

• **PARTICULATE MATTER IN INJECTIONS** <788>: See Table 4.

Table 4

Particle Size (µm)	Limit NMT (particles/container)
≥10	250
≥25	25
≥100	2
≥300	1

• **OSMOLALITY AND OSMOLARITY**, *Osmolality* <785>: 280–320 mOsmol/kg

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE**: Preserve in low-density polyethylene single-use ampuls, with a multilayer foil overwrap. Store at controlled room temperature.

• **LABELING**: The outer label indicates the dose and that the ampuls should be discarded if the solution is not colorless.

• USP REFERENCE STANDARDS (11)

USP Albuterol RS

USP Levalbuterol Hydrochloride RS

USP Levalbuterol Related Compound A RS

4-(2-*tert*-Butylamino-ethyl)-2-hydroxymethyl-phenol.

C₁₃H₂₁NO₂ 223.31

USP Levalbuterol Related Compound B RS

α[{(1,1-Dimethylethylamino)methyl]-4-hydroxy-3-methyl-benzenemethanol.

C₁₃H₂₁NO₂ 223.31

USP Levalbuterol Related Compound C RS

α[{(1,1-Dimethylethylamino)methyl]-4-hydroxy-3-(methoxymethyl)-benzenemethanol.

C₁₄H₂₃NO₃ 253.34

USP Levalbuterol Related Compound D RS

5-[2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxy-benzaldehyde;

Also known as 5-[2-[(1,1-Dimethylethyl)amino]methyl]-4-hydroxy-3-(methoxymethyl)-benzenemethanol.

C₁₃H₁₉NO₃ 237.29

[NOTE—This Reference Standard is available as the benzenesulfonic acid salt.]

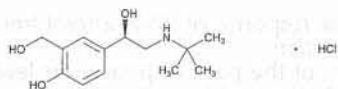
USP Levalbuterol Related Compound E RS

 α [(1,1-Dimethylethyl)amino]methyl]-3-(ethoxymethyl)-4-hydroxy-benzenemethanol.C₁₅H₂₅NO₃ 267.36

USP Levalbuterol Related Compound F RS

 α [(1,1-Dimethylethyl)amino]methyl]-4-(phenylmethoxy)-1,3-benzenedimethanol.C₂₀H₂₇NO₃ 329.43

Levalbuterol Hydrochloride



C₁₃H₂₁NO₃ · HCl 275.77
(R)- α 1-[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene- α , α' -diol hydrochloride [50293-90-8].

DEFINITION

Levalbuterol Hydrochloride contains NLT 98.0% and NMT 102.0% of levalbuterol hydrochloride (C₁₃H₂₁NO₃ · HCl), calculated on the anhydrous basis.

IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Diluted sample solution* corresponds to that of the levalbuterol peak of the *System suitability solution*, as obtained in the test for *Enantiomeric Purity*.

ASSAY

PROCEDURE

Solution A: Phosphoric acid in water (1 in 1000)

Solution B: Acetonitrile, methanol, phosphoric acid, and water (350:350:1:300)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	91.5	8.5
15	91.5	8.5
15.01	0	100
20	0	100
20.01	91.5	8.5
30	91.5	8.5

Diluent: *Solution A*

Standard solution: 100 μ g/mL of USP Levalbuterol Hydrochloride RS in *Diluent*

Sample solution: 100 μ g/mL of Levalbuterol Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 5500 theoretical plates

Tailing factor: NMT 2.3

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levalbuterol hydrochloride (C₁₃H₂₁NO₃ · HCl) in the portion of Levalbuterol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Levalbuterol Hydrochloride RS in the *Standard solution* (μ g/mL)

C_U = concentration of the *Sample solution* (μ g/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- HEAVY METALS, Method I** (231): NMT 10 ppm (Official 1-Jan-2018)

ORGANIC IMPURITIES

Solution A, Solution B, Diluent, and Sample solution:

Proceed as directed in the *Assay*.

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	70	30
50	28	72
50.01	0	100
55	0	100
55.01	100	0
70	100	0

System suitability solution: Prepare a solution containing the following in *Diluent*:

USP Levalbuterol Hydrochloride RS, 100 μ g/mL

USP Levalbuterol Related Compound A RS, 0.05 μ g/mL

USP Levalbuterol Related Compound B RS, 0.05 μ g/mL

USP Levalbuterol Related Compound C RS, 0.05 μ g/mL

USP Levalbuterol Related Compound D RS, 0.05 μ g/mL

USP Levalbuterol Related Compound E RS, 0.05 μ g/mL

USP Levalbuterol Related Compound F RS, 0.05 μ g/mL

USP Levalbuterol Related Compound H RS, 0.05 μ g/mL

Chromatographic system(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 220 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L1**Column temperature:** 45°**Flow rate:** 1 mL/min**Injection volume:** 50 μL**System suitability****Sample:** *System suitability solution***Suitability requirements****Resolution:** NLT 4.9 between levalbuterol and levalbuterol related compound A; NLT 1.5 between levalbuterol related compound B and levalbuterol related compound C**Tailing factor:** NMT 4.0 for levalbuterol**Analysis****Sample:** *Sample solution*

[NOTE—Integrate all peaks with an area greater than 0.05% of the area corresponding to the levalbuterol peak.]

Calculate the percentage of each impurity in the portion of Levalbuterol Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_T = sum of all the peak responses from the *Sample solution* F = relative response factor for each impurity (see *Table 3*)**Acceptance criteria:** See *Table 3*.**Table 3**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levalbuterol	1.0	—	—
Levalbuterol related compound A	1.2	1.0	0.1
Levalbuterol related compound H	1.3	1.0	0.15
Levalbuterol related compound B	1.5	1.0	0.10
Levalbuterol related compound C	1.6	1.0	0.15
Levalbuterol related compound D	1.7	3.0	0.05
Levalbuterol related compound E	2.1	1.0	0.1
Levalbuterol related compound F	3.5	1.2	0.10
Any individual unspecified impurity	—	—	0.10
Total impurities	—	—	0.5

• **ENANTIOMERIC PURITY****Mobile phase:** Acetonitrile, methanol, acetic acid, and triethylamine (500:500:3:1)**Diluent:** *Mobile phase***System suitability solution:** 0.10 mg/mL of USP Levalbuterol Hydrochloride RS and 0.04 mg/mL of USP Albuterol RS in *Diluent***Sample solution:** 0.8 mg/mL of Levalbuterol Hydrochloride in *Diluent***Diluted sample solution:** 0.1 mg/mL from the *Sample solution***Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 225 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L63**Flow rate:** 1 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *System suitability solution*

[NOTE—The relative retention times of levalbuterol and (S)-albuterol are 1.0 and 1.16, respectively.]

Suitability requirements**Resolution:** NLT 2.0 between levalbuterol and (S)-albuterol**Tailing factor:** NMT 2.2 for levalbuterol and (S)-albuterol**Relative standard deviation:** NMT 20% for (S)-albuterol for three injections**Analysis****Samples:** *Sample solution* and *Diluted sample solution*

Calculate the percentage of (S)-albuterol in the portion of Levalbuterol Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

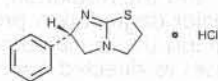
 r_U = peak response of (S)-albuterol from the *Sample solution* r_T = sum of the peak responses for levalbuterol and (S)-albuterol from the *Sample solution***Acceptance criteria:** NMT 0.2% of (S)-albuterol**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: The total aerobic bacterial count is less than 10^1 cfu/g. The total combined molds and yeasts count is less than 10^1 cfu/g. It meets the requirements of the tests for the absence of *Salmonella* species, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.
- **PH** (791): 4.5–5.5, in a 10-mg/mL solution
- **WATER DETERMINATION**, *Method 1c* <921>: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** <11>
 - USP Albuterol RS
 - USP Levalbuterol Hydrochloride RS
 - USP Levalbuterol Related Compound A RS
4-(2-*tert*-Butylamino-ethyl)-2-hydroxymethyl-phenol.
 $C_{13}H_{21}NO_2$ 223.31
 - USP Levalbuterol Related Compound B RS
α-[[[(1,1-Dimethylethyl)amino]methyl]-4-hydroxy-3-methyl-benzenemethanol.
 $C_{13}H_{21}NO_2$ 223.31
 - USP Levalbuterol Related Compound C RS
α-[[[(1,1-Dimethylethyl)amino]methyl]-4-hydroxy-3-(methoxymethyl)-benzenemethanol.
 $C_{14}H_{23}NO_3$ 253.34
 - USP Levalbuterol Related Compound D RS
5-[2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxy-benzaldehyde.
 $C_{13}H_{19}NO_3$ 237.29
 - USP Levalbuterol Related Compound E RS
α-[[[(1,1-Dimethylethyl)amino]methyl]-3-(ethoxymethyl)-4-hydroxy-benzenemethanol.
 $C_{15}H_{25}NO_3$ 267.36
 - USP Levalbuterol Related Compound F RS
α-[[[(1,1-Dimethylethyl)amino]methyl]-4-(phenylmethoxy)-1,3-benzenedimethanol.
 $C_{20}H_{27}NO_3$ 329.43
 - USP Levalbuterol Related Compound H RS
4-[2-(*tert*-Butylamino)-1-methoxyethyl]-2-(hydroxymethyl)phenol acetate.
 $C_{14}H_{23}NO_3 \cdot C_2H_4O_2$ 313.39

Levamisole Hydrochloride



$C_{11}H_{12}N_2S \cdot HCl$ 240.75

Imidazo[2,1-*b*]thiazole, 2,3,5,6-tetrahydro-6-phenyl-, monohydrochloride, (5*S*)-.

(-)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]thiazole monohydrochloride [16595-80-5].

» Levamisole Hydrochloride contains not less than 98.5 percent and not more than 101.0 percent of $C_{11}H_{12}N_2S \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers, protected from light.

USP Reference standards (11)—

USP Levamisole Hydrochloride RS

Completeness of solution (641)—A test solution of 500 mg of Levamisole Hydrochloride dissolved in 10 mL of water meets the requirements.

Color of solution—The test solution prepared for the test for *Completeness of solution* is colorless or not more intensely colored than a color matching fluid prepared by mixing 2.5 mL of *Matching Fluid F* (see *Color and Achromicity* (631)) with 97.5 mL of 0.12 N hydrochloric acid.

Identification—

A: The IR absorption spectrum of a potassium bromide dispersion of it, previously dried, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Levamisole Hydrochloride RS.

B: The color, size, and R_f value of the principal spot in the chromatogram of *Test solution B* obtained in the test for *Chromatographic purity*, when examined under short-wavelength UV light, correspond to the respective characteristics of the principal spot in the chromatogram of *Reference solution A* obtained in the test for *Chromatographic purity*.

C: A solution of it responds to the tests for *Chloride* (191).

Melting range (741): between 226° and 231°.

Light absorption—Its absorbance (see *Ultraviolet-Visible Spectroscopy* (857)) at 310 nm, determined in a 0.2 N methanolic hydrochloric acid solution containing 1 mg per mL using a 1-cm cell, is not more than 0.20.

Specific rotation (781S): between -121.5° and -128.0°.

Test solution: 50 mg per mL, in water.

pH (791): between 3.0 and 4.5, in a solution (1 in 20).

Loss on drying (731): Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals, Method I** (231): 0.001%. • (Official 1-Jan-2018)

Chromatographic purity—Prepare a solution of it in methanol containing 50 mg per mL (*Test solution A*). Dilute 1.0 mL of *Test solution A* to 10 mL with methanol, and mix (*Test solution B*). Prepare a solution of USP Levamisole Hydrochloride RS in methanol having a concentration of 5 mg per mL (*Reference solution A*). Dilute 1.0 mL of *Test solution B* to 20 mL with methanol, and mix (*Reference solution B*). Apply separate 10-μL portions of the four solutions on the starting line to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of

chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, acetone, and ammonium hydroxide (60:40:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and dry it at 105° for 15 minutes. Locate the spots on the plate by examination under short-wavelength UV light: any spot obtained from *Test solution A*, other than the one corresponding to levamisole, does not exceed, in size or intensity, the principal spot obtained from *Reference solution B*, corresponding to not more than 0.5% of any individual impurity. Expose the plate to iodine vapor in a closed chamber for 15 minutes, and locate the spots on the plate: any spot obtained from *Test solution A*, other than the one corresponding to levamisole, does not exceed, in size or intensity, the principal spot obtained from *Reference solution B*, corresponding to not more than 0.5% of any individual impurity, and the total of all impurities found does not exceed 1.0%.

Assay—Dissolve about 200 mg of Levamisole Hydrochloride, accurately weighed, in 30 mL of alcohol. Add 5.0 mL of 0.01 N hydrochloric acid, and titrate with 0.1 N sodium hydroxide VS, determining the two inflection points potentiometrically. Determine the volume, in mL, of 0.1 N sodium hydroxide consumed between the two inflection points. Each mL of 0.1 N sodium hydroxide consumed is equivalent to 24.08 mg of $C_{11}H_{12}N_2S \cdot HCl$.

Levamisole Hydrochloride Tablets

» Levamisole Hydrochloride Tablets contain an amount of Levamisole Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of levamisole ($C_{11}H_{12}N_2S$).

Packaging and storage—Preserve in well-closed containers.

Labeling—Label it to state both the content of the active moiety and the content of the salt used in formulating the article.

USP Reference standards (11)—

USP Levamisole Hydrochloride RS

Identification—

A: The retention time of the major peak for levamisole in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: The R_f value of the principal spot obtained from *Test solution B* in the *Chromatographic purity* test corresponds to that from *Standard solution A*.

Dissolution (711)—

Medium: 0.01 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Procedure—Determine the amount of levamisole ($C_{11}H_{12}N_2S$) dissolved by employing UV absorption at the wavelength of maximum absorbance at about 214 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Levamisole Hydrochloride RS in the same *Medium*.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{11}H_{12}N_2S$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Chromatographic purity—

Test solution A—Transfer an amount of powdered Tablets, equivalent to 100 mg of levamisole, to a glass test tube. Add 5.0 mL of methanol, shake for 2 minutes, and filter.

Test solution B—Dilute 1.0 mL of *Test solution A* to 10 mL with methanol, and mix.

Standard solution A—Prepare a solution of USP Levamisole Hydrochloride RS in methanol having a concentration of 2.4 mg per mL (equivalent to 2.0 mg of levamisole per mL).

Standard solution B—Dilute 1.0 mL of *Standard solution A* to 20 mL with methanol, and mix.

Procedure—Apply separate 10-μL portions of *Test solutions A* and *B* and *Standard solutions A* and *B* to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of toluene, acetone, and ammonium hydroxide (60:40:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and dry the plate at 105° for 15 minutes. Locate the spots on the plate by examination under short-wavelength UV light: any spot obtained from *Test solution A*, other than that of levamisole, does not exceed, in size or intensity, the principal spot obtained from *Standard solution B*, corresponding to not more than 0.5% of any individual impurity. Expose the plate to iodine vapor in a closed chamber for 15 minutes, and locate the spots on the plate: any spot obtained from *Test solution A*, other than that of levamisole, does not exceed, in size or intensity, the principal spot obtained from *Standard solution B*, corresponding to not more than 0.5% of any individual impurity.

Assay—

Solution A—Prepare a 0.75% solution of monobasic ammonium phosphate in water, and adjust with diisopropylamine to a pH of 7.

Solution B—Use acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Transfer about 20 mg of USP Levamisole Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add 10 mL of water, and swirl to dissolve. Dilute with methanol to volume, and mix to obtain a solution having a known concentration of about 0.2 mg of USP Levamisole Hydrochloride RS per mL.

Resolution solution—Dissolve 20 mg of Levamisole Hydrochloride in 5 mL of 0.1 N sodium hydroxide, and heat at 100° in a closed vial for 5 hours. Allow to cool, and dilute 1 mL of the solution to 25 mL with methanol.

Assay preparation—Transfer an accurately counted number of Tablets, equivalent to about 150 mg of levamisole (C₁₁H₁₂N₂S), to a 100-mL volumetric flask. Add 25 mL of water, and shake by mechanical means for 30 minutes. Dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 10-cm column that contains 3-μm packing L1. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

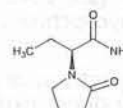
Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–5	80→20	20→80	linear gradient
5–7	20	80	isocratic
7–8	20→80	80→20	linear gradient
8–12	80	20	isocratic

Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are 1.0 for levamisole and about 1.3 for the major degradation product; and the resolution, *R*, between levamisole and the major degradation product is not less than 6.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor, *k'*, is not less than 3.0; the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of levamisole (C₁₁H₁₂N₂S) in the Tablets taken by the formula:

$$(204.29 / 240.75)(1000C)(r_U / r_S)$$

in which 204.29 and 240.75 are the molecular weights of levamisole and levamisole hydrochloride, respectively; *C* is the concentration, in mg per mL, of USP Levamisole Hydrochloride RS in the *Standard preparation*; and *r_U* and *r_S* are the levamisole peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Levetiracetam

C₈H₁₄N₂O₂ 170.21
1-Pyrrolidineacetamide, α-ethyl-2-oxo-, (αS)-;
(-)-(S)-α-Ethyl-2-oxo-1-pyrrolidineacetamide [102767-28-2].

DEFINITION

Levetiracetam contains NLT 98.0% and NMT 102.0% of levetiracetam (C₈H₁₄N₂O₂), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- A. INFRARED ABSORPTION (197K)**
- B.** The retention time of the major peak of the *Identification solution* corresponds to that of the levetiracetam *S*-enantiomer from the *System suitability solution*, as obtained in the test for *Limit of Levetiracetam R-Enantiomer*.

ASSAY**PROCEDURE**

Buffer: 2.7 g/L of monobasic potassium phosphate in water. Adjust with 2% aqueous potassium hydroxide (w/v) to a pH of 5.5.

Solution A: Acetonitrile and *Buffer* (1:19)

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
20	71	29

System suitability solution: 0.2 mg/mL of USP Levetiracetam RS and 0.08 mg/mL of USP Levetiracetam Related Compound A RS in *Solution A*. Prepare by first dissolving the required amount of USP Levetiracetam RS in a suitable volumetric flask. Add 10% of the flask vol-

ume of 0.1 N potassium hydroxide. Let the mixture react at room temperature for about 15 min, and then neutralize by adding 0.1 N hydrochloric acid at 10% of the flask volume. Add the required amount of USP Levetiracetam Related Compound A RS, sonicate to dissolve, dilute with *Solution A* to volume, and mix.

[NOTE—Levetiracetam related compound A is included for peak identification purposes.]

Standard solution: 0.1 mg/mL of USP Levetiracetam RS in *Solution A*

Sample solution: 0.1 mg/mL of Levetiracetam in *Solution A*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 0.9 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Relative standard deviation: NMT 1.0%, for the levetiracetam peak

[NOTE—If system suitability criteria cannot be met, it is recommended that the column temperature be maintained at 20° to stabilize the system.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levetiracetam ($C_8H_{14}N_2O_2$) in the portion of Levetiracetam taken:

$$\text{Result} = [(r_U/r_S) \times (C_S/C_U) \times 100] - F$$

r_U = peak response of levetiracetam from the *Sample solution*

r_S = peak response of levetiracetam from the *Standard solution*

C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)

C_U = concentration of Levetiracetam in the *Sample solution* (mg/mL)

F = percentage of levetiracetam *R*-enantiomer from the test for *Limit of Levetiracetam R-Enantiomer*

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS**, *Method II* (231): 20 ppm (Official 1-Jan-2018)

• LIMIT OF LEVETIRACETAM *R*-ENANTIOMER

Mobile phase: *n*-Hexane and dehydrated alcohol (80:20)

System suitability solution: 0.1 mg/mL of USP Levetiracetam Racemic Mixture RS in *Mobile phase*

Standard solution: 0.05 mg/mL of USP Levetiracetam RS in *Mobile phase*

Sample solution: 10 mg/mL of Levetiracetam in *Mobile phase*

Identification solution: 0.05 mg/mL of Levetiracetam from *Sample solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 10-µm packing L51

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Identification solution*

[NOTE—The relative retention times for levetiracetam *R*-enantiomer and levetiracetam *S*-enantiomer are 0.55 and 1.0, respectively. Use the chromatogram from the *Identification solution* for *Identification test B*.]

Suitability requirements

Resolution: NLT 4.0 between the *R*- and *S*-enantiomers, *System suitability solution*. [NOTE—If a loss of resolution (less than 4.0) is observed, it is recommended that the column temperature be maintained at 25° to stabilize the system.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levetiracetam *R*-enantiomer in the portion of Levetiracetam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levetiracetam *R*-enantiomer from the *Sample solution*

r_S = peak response of levetiracetam from the *Standard solution*

C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)

C_U = concentration of Levetiracetam in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.8%

• LIMIT OF LEVETIRACETAM RELATED COMPOUND B

[NOTE—Perform this test only if levetiracetam related compound B is a known process impurity.]

Buffer: 1.22 g of sodium 1-decanesulfonate in 1 L of water containing about 1.3 mL of phosphoric acid. Adjust with 20% (w/v) potassium hydroxide to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (3:17)

System suitability solution: 2 mg/mL of USP Levetiracetam Related Compound B RS in *Mobile phase*

Standard solution: 0.002 mg/mL of USP Levetiracetam Related Compound B RS in *Mobile phase*

Sample solution: 2.0 mg/mL of Levetiracetam in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.0 mL/min

Injection volumes

System suitability: 10 µL

Analysis: 50 µL

System suitability

Sample: *System suitability solution*

[NOTE—The retention time for levetiracetam related compound B is 9 min.]

Suitability requirements

Tailing factor: NMT 3.0. [NOTE—If a significant tailing of the levetiracetam related compound B peak is observed (greater than 3.0), it is recommended that the column temperature be maintained at 27° to stabilize the system.]

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levetiracetam related compound B in the portion of Levetiracetam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response of levetiracetam related compound B from the *Sample solution*
 r_S = peak response of levetiracetam related compound B from the *Standard solution*
 C_S = concentration of USP Levetiracetam Related Compound B RS in the *Standard solution* (mg/mL)
 C_U = concentration of Levetiracetam in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of levetiracetam related compound B free base, 102.1
 M_{r2} = molecular weight of levetiracetam related compound B, 138.6

Acceptance criteria: NMT 0.10%

[NOTE—The amount of levetiracetam related compound B measured is to be included in the total impurities in the test for *Organic Impurities*.]

• ORGANIC IMPURITIES

Buffer, Solution A, Solution B, Mobile phase, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Standard solution: 0.005 mg/mL of USP Levetiracetam RS in *Solution A*

Sample solution: 5 mg/mL of Levetiracetam in *Solution A*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Levetiracetam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- r_U = peak response of each impurity from the *Sample solution*
 r_S = peak response of levetiracetam from the *Standard solution*
 C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)
 C_U = concentration of Levetiracetam in the *Sample solution* (mg/mL)
 F = relative response factor (see *Table 2*)

[NOTE—Disregard any peak with a relative retention time of 0.19 or less.]

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Pyridin-2-ol ^a	0.37	1.0	0.025
Levetiracetam acid ^b	0.62	1.2	0.3
Levetiracetam	1.00	—	—
Levetiracetam related compound A ^c	1.25	0.35	0.05
Any individual unspecified impurity	—	1.0	0.05
Total impurities	—	—	0.4

^a Not included in the total impurities limit.

^b (S)-2-(2-Oxopyrrolidin-1-yl)butanoic acid. Included in the total impurities limit.

^c (S)-N-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide. Included in the total impurities limit only if levetiracetam related compound B is a known process impurity.

SPECIFIC TESTS

- **WATER DETERMINATION** (921), *Method 1a*: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in well-closed containers, and store at room temperature.

• USP REFERENCE STANDARDS (11)

USP Levetiracetam RS

USP Levetiracetam Racemic Mixture RS

A 1:1 mixture of:

Levetiracetam S-enantiomer-(2S)-2-(2-oxopyrrolidin-1-yl)butanamide;

Levetiracetam R-enantiomer (2R)-2-(2-oxopyrrolidin-1-yl)butanamide.

USP Levetiracetam Related Compound A RS

(S)-N-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.

$C_8H_{15}ClN_2O_2$ 206.67

USP Levetiracetam Related Compound B RS

(S)-2-Aminobutanamide hydrochloride.

$C_4H_{10}N_2O \cdot HCl$ 138.6

Levetiracetam Injection

DEFINITION

Levetiracetam Injection is a sterile solution of levetiracetam in Water for Injection and contains NLT 90.0% and NMT 110.0% of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$). Levetiracetam Injection may contain buffering and isotonicity agents. Levetiracetam Injection contains no antimicrobial agent.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Buffer: 1.0 g/L of anhydrous dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.0.

Mobile phase: Acetonitrile and *Buffer* (6:94)

Diluent: Acetonitrile and water (6:94)

System suitability solution: Solution containing levetiracetam and levetiracetam acid prepared from a solution of 0.2 mg/mL of USP Levetiracetam RS as follows. Dissolve the required amount of USP Levetiracetam RS in 10% of the final volume of 0.1 N potassium hydroxide. Let the mixture react at room temperature for about 15 min, then neutralize by adding 10% of the flask volume of 0.1 N hydrochloric acid. Dilute with *Diluent* to volume.

Standard solution: 100 µg/mL of USP Levetiracetam RS in *Diluent*. Sonication may be used to aid in dissolution if necessary.

Sample solution: Nominally 100 µg/mL of levetiracetam from NLT 2 mL of Injection in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 1.5 times the retention time of levetiracetam

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—Identify the peaks using the relative retention times given in *Table 1*.]

Suitability requirements

Tailing factor: NMT 2.0 for the levetiracetam peak, *System suitability solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of levetiracetam from the *Sample solution*
 r_S = peak response of levetiracetam from the *Standard solution*
 C_S = concentration of USP Levetiracetam RS in the *Standard solution* ($\mu\text{g/mL}$)
 C_U = nominal concentration of levetiracetam in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0%

IMPURITIES• **ORGANIC IMPURITIES**

Buffer, Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 0.1 $\mu\text{g/mL}$ of USP Levetiracetam RS in Diluent

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—Identify the peaks using the relative retention times in Table 1.]

Suitability requirements

Tailing factor: NMT 2.0 for the levetiracetam peak, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levetiracetam acid and any other unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of levetiracetam acid or any individual unspecified degradation product from the *Sample solution*
 r_S = peak response of levetiracetam from the *Standard solution*
 C_S = concentration of USP Levetiracetam RS in the *Standard solution* ($\mu\text{g/mL}$)
 C_U = nominal concentration of levetiracetam in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levetiracetam acid ^a	0.4	0.3
Levetiracetam	1.0	—
Any individual unspecified degradation product	—	0.10
Total impurities	—	1.00

^a (S)-2-(2-Oxopyrrolidin-1-yl)butanoic acid.

SPECIFIC TESTS

- **PH (791):** 5.0–6.0
- **BACTERIAL ENDOTOXINS TEST (85):** Contains NMT 0.175 USP Endotoxin Units/mg of levetiracetam

- **STERILITY TESTS (71):** Meets the requirements when tested as directed for *Aqueous Solutions* under *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed Type I glass vials. Store at controlled room temperature.
- **LABELING:** Label the article to indicate that the Injection is to be diluted prior to administration.
- **USP REFERENCE STANDARDS (11)**
USP Endotoxin RS
USP Levetiracetam RS

Levetiracetam Oral Solution**DEFINITION**

Levetiracetam Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY• **PROCEDURE**

Solution A: Dilute 1 mL of phosphoric acid with water to 1 L.

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	92	8
6	92	8
7	40	60
10	40	60
11	92	8
15	92	8

Standard solution: 1.0 mg/mL of USP Levetiracetam RS in *Solution A*

Sample solution: Nominally 1.0 mg/mL of levetiracetam prepared as follows. Transfer a suitable volume of the Oral Solution to a suitable volumetric flask to obtain 1.0 mg/mL final concentration of levetiracetam. Add 60% of the flask volume of *Solution A*, and sonicate at room temperature for 5 min with intermittent shaking. Allow the solution to cool, and dilute with *Solution A* to volume. Pass a portion of the solution under test through a suitable filter.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitabilitySample: *Standard solution***Suitability requirements**

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of levetiracetam (C₈H₁₄N₂O₂) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of levetiracetam from the *Sample solution* r_S = peak response of levetiracetam from the *Standard solution* C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL) C_U = nominal concentration of levetiracetam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES**• ORGANIC IMPURITIES****Solution A:** Dilute 2 mL of phosphoric acid with water to 1 L.**Solution B:** Acetonitrile**Diluent:** Acetonitrile and *Solution A* (5:95)**Mobile phase:** See *Table 2*.**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7	95	5
20	90	10
30	75	25
35	50	50
40	50	50
41	100	0
50	100	0

System suitability solution: 0.2 mg/mL of USP Levetiracetam RS and 0.1 mg/mL of USP Levetiracetam Related Compound A RS in *Diluent* prepared as follows. Dissolve the required amount of USP Levetiracetam RS in 10% of the final volume of 0.1 N potassium hydroxide. Let the mixture react at room temperature for about 15 min, and then neutralize by adding 0.1 N hydrochloric acid at 10% of the flask volume. Add the required amount of USP Levetiracetam Related Compound A RS, sonicate to dissolve, and dilute with *Diluent* to volume. [NOTE—This solution contains levetiracetam, levetiracetam acid, and levetiracetam related compound A.]

Standard solution: 3 μg/mL of USP Levetiracetam RS in *Solution A*

Sample solution: Nominally 2 mg/mL of levetiracetam prepared as follows. Transfer a suitable volume of the Oral Solution to a suitable volumetric flask. Add 60% of the flask volume of *Solution A*, and sonicate at room temperature for 5 min with intermittent shaking. Allow the solution to cool, and dilute with *Solution A* to volume. Pass a portion of the solution through a suitable filter.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitabilitySamples: *System suitability solution* and *Standard solution***Suitability requirements**Resolution: NLT 2.0 between levetiracetam related compound A and levetiracetam acid, *System suitability solution*Tailing factor: NMT 2.0, *Standard solution*Relative standard deviation: NMT 5.0%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak response of the impurity from the *Sample solution* r_S = peak response of levetiracetam from the *Standard solution* C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL) C_U = nominal concentration of levetiracetam in the *Sample solution* (mg/mL) F = relative response factor for each impurity (see *Table 3*)Acceptance criteria: See *Table 3*.**Table 3**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levetiracetam	1.00	—	—
Levetiracetam related compound A ^{a,b}	1.38	—	—
Levetiracetam acid ^c	1.46	0.92	0.3
Any individual unspecified degradation product	—	1.0	0.10
Total impurities	—	—	1.0

^a (S)-N-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.^b This is a process impurity and included for peak identification purposes only.^c (S)-2-(2-Oxopyrrolidin-1-yl)butanoic acid.**SPECIFIC TESTS****• PH (791):** 4.8–6.3

• MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62): The total aerobic microbial count does not exceed 10² cfu/mL. The total yeasts and molds count does not exceed 10¹ cfu/mL. It meets the requirement of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in light-resistant containers. Store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Levetiracetam RS

USP Levetiracetam Related Compound A RS

(S)-N-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.

C₈H₁₅ClN₂O₂ 206.67

Levetiracetam Tablets

DEFINITION

Levetiracetam Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197K), (197A)

Standard solution: 1 mg/mL solution of USP Levetiracetam RS in solution prepared as follows. Transfer a suitable quantity of USP Levetiracetam RS to a suitable volumetric flask. Add 70% of the flask volume of acetone. Sonicate for 15 min. Dilute with acetone to volume.

Standard: Pass 10 mL of the *Standard solution* through a membrane filter of 0.45- μ m pore size. Evaporate acetone from the filtrate completely to form crystals. Scratch the crystals. Weigh 2–4 mg of the residue and 200 mg of KBr in a mortar and pestle. Mix and grind well, and prepare the KBr pellet.

Sample solution: Transfer an amount of finely powdered Tablets (NLT 20) equivalent to 250 mg of levetiracetam to a 50-mL volumetric flask. Add 35 mL of acetone. Sonicate for 15 min. Dilute with acetone to volume.

Sample: Pass 10 mL of the *Sample solution* through a membrane filter of 0.45- μ m pore size. Evaporate acetone from the filtrate completely to form crystals. Scratch the crystals. Weigh 2–4 mg of the residue and 200 mg of KBr in a mortar and pestle. Mix and grind well, and prepare the KBr pellet.

Analysis: Record the spectra of the *Standard* and *Sample* between 4000 cm^{-1} and 650 cm^{-1} .

Acceptance criteria: The spectrum of the *Sample* corresponds to that of the *Standard*.

- **B.** The retention time of the major peak of the *Sample* solution corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer: 1.4 g/L of monobasic potassium phosphate and 0.6 g/L of sodium 1-heptanesulfonate, adjusted with phosphoric acid to a pH of 2.8

Mobile phase: Acetonitrile and *Buffer* (8:92)

Diluent: Acetonitrile and water (20:80)

Standard solution: 0.35 mg/mL of USP Levetiracetam RS in *Diluent*. Sonication may be used to aid dissolution.

Sample solution: Nominally 0.4 mg/mL of levetiracetam from NLT 20 Tablets, finely crushed, in *Diluent*. Sonication may be used to aid dissolution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 25-cm; 4- μ m packing L1

Flow rate: 2 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levetiracetam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: See *Table 1*.

Table 1

Tablet Strength (mg/Tablet)	Time (min)
250	15
500	15
750	15
1000	30

Buffer: 6.8 g/L of monobasic potassium phosphate, adjusted with dilute potassium hydroxide to a pH of 5.6

Mobile phase: Acetonitrile and *Buffer* (15:85)

Standard solution: ($L/1000$) mg/mL in *Medium*, where L is the Tablet label claim, in mg

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1.2 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances

NLT 70% (Q) of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in 15 min for Tablets labeled to contain 250, 500, or 750 mg; NLT 80% (Q) of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in 30 min for Tablets labeled to contain 1000 mg.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution* Test 2.

Medium: Water; 900 mL, deaerate, if necessary

Apparatus 2: 50 rpm

Time: 15 min

Buffer: 1.36 g/L of monobasic potassium phosphate, adjusted with 10% potassium hydroxide to a pH of 5.0

Mobile phase: Acetonitrile and *Buffer* (10:90)

Standard solution: 54 μ g/mL of USP Levetiracetam RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute an aliquot with *Medium* to obtain a concentration similar to that of the *Standard solution*.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of levetiracetam (C₈H₁₄N₂O₂) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL) L = label claim (mg/Tablet) D = dilution factor of the *Sample solution* V = volume of *Medium*, 900 mLTolerances: NLT 80% (Q) of the labeled amount of levetiracetam (C₈H₁₄N₂O₂) is dissolved.**Test 3:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution* Test 3.**Medium:** Water; 900 mL**Apparatus 2:** 50 rpm**Time:** 30 min**Buffer, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and Analysis:** Proceed as directed for *Test 1*.Tolerances: NLT 80% (Q) of the labeled amount of levetiracetam (C₈H₁₄N₂O₂) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES• **ORGANIC IMPURITIES****Buffer:** 6.8 g/L of monobasic potassium phosphate and 0.85 g/L of sodium 1-heptanesulfonate, adjusted with phosphoric acid to a pH of 2.8**Mobile phase:** Acetonitrile and *Buffer* (5:95)**System suitability solution:** 3.6 μg/mL of USP Levetiracetam RS and 3.6 μg/mL of USP Levetiracetam Related Compound B RS in *Mobile phase***Standard solution:** 3.6 μg/mL of USP Levetiracetam RS in *Mobile phase***Sample solution:** Equivalent to 1.2 mg/mL of levetiracetam from NLT 20 Tablets, finely crushed, in *Mobile phase*. [NOTE—Sonicate if necessary, and centrifuge the solution before passing through a suitable filter.]**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 25-cm; 4-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitabilitySamples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between levetiracetam related compound B and levetiracetam, *System suitability solution***Tailing factor:** NMT 2.0, *Standard solution***Relative standard deviation:** NMT 10.0%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_S = peak response of levetiracetam from the *Standard solution* C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL) C_U = nominal concentration of levetiracetam in the *Sample solution* (mg/mL) F = relative response factor (see *Table 2*)Acceptance criteria: See *Table 2*.**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levetiracetam related compound B ^a	0.54	—	—
Levetiracetam	1.0	—	—
Levetiracetam related compound A ^{a,b}	1.7	—	—
Levetiracetam acid ^c	2.1	0.79	0.3
Any individual unspecified degradation product	—	1.0	0.1
Total impurities	—	—	0.6

^a These impurities are listed for information only; they are process impurities, which are controlled in the drug substance.^b (S)-N-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.^c (S)-2-(2-Oxopyrrolidine-1-yl)butanoic acid.**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
USP Levetiracetam RS
USP Levetiracetam Related Compound B RS
(S)-2-Aminobutanamide hydrochloride.
C₄H₁₀N₂O · HCl 138.60

Levetiracetam Extended-Release Tablets**DEFINITION**Levetiracetam Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of levetiracetam (C₈H₁₄N₂O₂).**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE****Buffer:** 1.4 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.**Mobile phase:** Acetonitrile and *Buffer* (10:90)**Standard stock solution:** 1.0 mg/mL of USP Levetiracetam RS prepared as follows. Weigh a suitable quantity of the Reference Standard into a volumetric

flask. Add *Mobile phase* to fill 60% of flask volume and tetrahydrofuran to fill 4% of flask volume. Sonicate in cool water to dissolve. Equilibrate to room temperature. Dilute with *Mobile phase* to volume.

Standard solution: 0.08 mg/mL of USP Levetiracetam RS in *Mobile phase* from *Standard stock solution*. Pass a portion of the solution through a suitable filter of 0.45- μ m pore size.

Sample stock solution: Nominally ($L/100$) mg/mL of levetiracetam from NLT 5 Tablets prepared as follows, where L is the label claim in mg/Tablet. Transfer the Tablets to a volumetric flask containing tetrahydrofuran to fill about 5% of flask volume. Stir for 30 min, and allow to stand for 5 min. Sonicate for 20 min with intermittent shaking. Add *Mobile phase* to fill 80% of final volume, and sonicate in cold water for 20 min with intermittent shaking. Add methanol to fill 10% of flask volume. Dilute with *Mobile phase* to volume. Centrifuge for 15 min, and pass a portion of the solution through a suitable filter of 0.2- μ m pore size.

Alternatively, the *Sample stock solution*, having a nominal concentration of 3 mg/mL of levetiracetam, may be prepared as follows. Finely grind NLT 10 Tablets, and transfer an amount equivalent to 750 mg of levetiracetam to a suitable volumetric flask. Add 18% of the flask volume of acetonitrile. Sonicate for 10 min followed by shaking using a mechanical shaker for 10 min. Add 18% of the flask volume of water, and shake for 15 min using a mechanical shaker. Allow the sample to equilibrate to room temperature, and dilute with a mixture of acetonitrile and water (50:50) to volume. Pass a portion of the solution through a suitable filter of 0.45- μ m pore size.

Sample solution: Nominally 0.08 mg/mL of levetiracetam in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Temperatures

Column: 30°

Autosampler: 10°

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

Run time: 3 times the retention time of levetiracetam

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levetiracetam from the *Sample solution*

r_S = peak response of levetiracetam from the *Standard solution*

C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levetiracetam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• DISSOLUTION (711)

Test 1

Buffer A: Dissolve 6.8 g of potassium dihydrogen phosphate and 0.2 g of sodium hydroxide in 1 L of water. If necessary, adjust with 1 N sodium hydroxide to a pH of 6.0.

Medium: *Buffer A*; 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, 4, and 8 h

Buffer B: 1.4 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Acetonitrile and *Buffer B* (10:90)

Standard stock solution: 1.7 mg/mL of USP Levetiracetam RS in water. Sonication may be used to aid in dissolution.

Standard solution: ($L/900$) mg/mL of USP Levetiracetam RS in *Medium* from *Standard stock solution*, where L is the label claim in mg/Tablet. Pass a portion through a suitable filter of 0.45- μ m pore size.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Temperatures

Column: 30°

Autosampler: 10°

Flow rate: 1.5 mL/min

Injection volume: 5 μ L

Run time: 2 times the retention time of levetiracetam

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_i , of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point i :

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

C_i = concentration of levetiracetam in the portion of sample withdrawn at the specified time point (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)
 V_s = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See Table 1.

Table 1

Time Point (i)	Time (h)	Amount Dissolved	
		500 mg/ Tablet (%)	750 mg/ Tablet (%)
1	1	25–45	33–53
2	2	45–65	45–65
3	4	60–80	65–85
4	8	NLT 80	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* (711), *Acceptance Table 2*.

Test 2: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 2*.

Buffer A: Dissolve 6.8 g of potassium dihydrogen phosphate and 0.2 g of sodium hydroxide in 1 L of water. If necessary, adjust with 1 N sodium hydroxide to a pH of 6.0.

Medium: Buffer A; 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, 4, and 8 h

Buffer B: 2.82 g/L of potassium dihydrogen phosphate in water

Mobile phase: Acetonitrile and Buffer B (5:95). Adjust with phosphoric acid to a pH of 2.0.

Standard solution: ($L/900$) mg/mL of USP Levetiracetam RS in *Medium*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Columns

Guard: 4.6-mm \times 1-cm, 4.6-mm \times 2-cm, or 4.0-mm \times 2-cm; 5- μ m packing L1

Analytical: 4.6-mm \times 5-cm; 5- μ m packing L1

Flow rate: 0.8 mL/min

Injection volume: 10 μ L

Run time: 2 times the retention time of levetiracetam

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.5% for five replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_s , of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point i :

$$\text{Result}_i = (r_u/r_s) \times C_s$$

r_u = peak response from the *Sample solution*
 r_s = peak response from the *Standard solution*
 C_s = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_s)] + (C_1 \times V_s)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_s)]] + [(C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times [V - (3 \times V_s)]] + [(C_3 + C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

C_i = concentration of levetiracetam in *Medium* in the portion of sample withdrawn at time point i (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_s = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See Table 2.

Table 2

Time Point (i)	Time (h)	Amount Dissolved	
		500 mg/ Tablet (%)	750 mg/ Tablet (%)
1	1	22–42	16–36
2	2	39–59	30–50
3	4	62–82	50–70
4	8	NLT 80	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* (711), *Acceptance Table 2*.

Test 3: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 3*.

Buffer A: Dissolve 6.8 g of monobasic sodium phosphate dihydrate and 0.5 g of sodium hydroxide in 1 L of water. Adjust to a pH of 6.0.

Medium: Buffer A; 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, 4, and 8 h

Buffer B: 7.8 g/L of potassium dihydrogen phosphate in water. Adjust with sodium hydroxide to a pH of 5.6.

Mobile phase: Acetonitrile and Buffer B (15:85)

Standard solution: ($L/900$) mg/mL of USP Levetiracetam RS in *Medium*, where L is the label claim in mg/Tablet

Sample solution: Centrifuge a portion of the solution under test.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

Run time: 2 times the retention time of levetiracetam

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Relative standard deviation: NMT 2.0% for six replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the concentration, C_i , of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point i :

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times (V - V_S)) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = [(C_3 \times [V - (2 \times V_S)]) + [(C_2 + C_1) \times V_S]] \times (1/L) \times 100$$

$$\text{Result}_4 = [(C_4 \times [V - (3 \times V_S)]) + [(C_3 + C_2 + C_1) \times V_S]] \times (1/L) \times 100$$

C_i = concentration of levetiracetam in *Medium* in the portion of sample withdrawn at time point i (mg/mL)
 V = volume of *Medium*, 900 mL
 L = label claim (mg/Tablet)
 V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See Table 3.

Table 3

Time Point (i)	Time (h)	Amount Dissolved	
		500 mg/ Tablet (%)	750 mg/ Tablet (%)
1	1	42–62	35–55
2	2	59–79	50–70
3	4	78–98	70–90
4	8	NLT 80	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* (711), *Acceptance Table 2*.

Test 4: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 4*.

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with sodium hydroxide to a pH of 6.0.

Medium: *Buffer*, 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, 4, and 8 h

Standard solution: (L/900) mg/mL of USP Levetiracetam RS in *Medium*, where L is the label claim in mg/Tablet

Sample solution: Pass a suitable portion of the solution under test through a suitable filter of 0.45- μ m pore size. Discard the first 3 mL of the filtrate. Dilute a known volume of the remaining filtrate quantitatively with *Medium*.

Blank: *Medium*

Instrumental conditions

Mode: UV

Analytical wavelength: 210 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_i , of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point i :

$$\text{Result}_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = [(C_3 \times V) + [(C_2 + C_1) \times V_S]] \times (1/L) \times 100$$

$$\text{Result}_4 = [(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]] \times (1/L) \times 100$$

C_i = concentration of levetiracetam in the portion of sample withdrawn at the specified time point (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See Table 4.

Table 4

Time Point (i)	Time (h)	Amount Dissolved	
		500 mg/ Tablet (%)	750 mg/ Tablet (%)
1	1	22–42	16–36
2	2	39–59	30–50
3	4	62–82	50–70
4	8	NLT 80	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* (711), *Acceptance Table 2*.

Test 5: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium: pH 6.0 phosphate buffer (6.8 g/L of monobasic potassium phosphate in water. Adjust with sodium hydroxide to a pH of 6.0.); 900 mL

Apparatus 1: 100 rpm

Times: 1, 4, 8, and 12 h

Buffer: 2.7 g/L of monobasic potassium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (10:90)

Standard stock solution: 2.8 mg/mL of USP Levetiracetam RS in *Medium* prepared as follows. Transfer a suitable quantity of USP Levetiracetam RS to a suitable volumetric flask. Dissolve in 20% of the flask volume of methanol. Dilute with *Medium* to volume.

Standard solution: (L/900) mg/mL of USP Levetiracetam RS in *Medium* from *Standard stock solution*, where L is the label claim in mg/Tablet

Sample solution: At each time point withdraw 1 mL of the solution under test, and pass it through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L11

Flow rate: 1 mL/min

Injection volume: 10 μ L

Run time: 2 times the retention time of levetiracetam

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 4000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0% for five replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: See Table 5.

Table 5

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 40
2	4	55–80
3	8	NLT 75
4	12	NLT 85

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* (711), *Acceptance Table 2*.

Test 6: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium: pH 6.0 phosphate buffer (6.9 g of monobasic sodium phosphate, and 0.23 g of sodium hydroxide in 1 L of water. Adjust with sodium hydroxide or phosphoric acid to a pH of 6.0.); 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, 4, and 8 h

Mobile phase: Acetonitrile and water (10:90)

Standard solution: 0.5 mg/mL of USP Levetiracetam RS in *Medium* prepared as follows. Transfer a suitable quantity of USP Levetiracetam RS to a suitable volumetric flask. Add 4% of the flask volume of methanol and 60% of the flask volume of the *Medium*. Sonicate for NLT 5 min. Dilute with *Medium* to volume.

Sample solution: At the end of specified time interval, withdraw a known volume of the solution from the dissolution vessel. Pass a suitable portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 5-cm; 5- μ m packing L1

Column temperature: 30°

Flow rate: 0.9 mL/min

Injection volume: 10 μ L

Run time: 2 times the retention time of levetiracetam

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_i , of levetiracetam ($C_8H_{14}N_2O_2$) in *Medium* (mg/mL) after time point *i*:

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_3)] + (C_1 \times V_3)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_3)]] + [(C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times [V - (3 \times V_3)]] + [(C_3 + C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

C_i = concentration of levetiracetam in *Medium* in the portion of sample withdrawn at time point *i* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_3 = volume of the *Sample solution* withdrawn from the solution under test (mL)

Tolerances: See Table 6.

Table 6

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	25–45
2	2	45–65
3	4	60–80
4	8	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to *Dissolution* (711), *Acceptance Table 2*.

Test 7: If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 7*.

Medium: Acetate buffer, pH 4.5, prepared as follows. Dissolve 3.0 g of sodium acetate in 1 L of water and add 1.4 mL of glacial acetic acid. Adjust with 5 N sodium hydroxide or glacial acetic acid to a pH of 4.5; 230 mL

Apparatus 3: 15 dips per min, with suitable screens

For 500-mg Tablets: 1, 2, 4, and 8 h

For 750-mg Tablets: 1, 2, 4, and 10 h

Buffer: 13.6 g/L of monobasic potassium phosphate in water. Adjust with 5 N sodium hydroxide to a pH of 6.0.

Mobile phase: Methanol and Buffer (15:85)

Standard solution: 0.55 mg/mL of USP Levetiracetam RS in Buffer A. Sonication may be used to aid in dissolution.

Sample solution: Pass a suitable portion of the solution under test through a suitable filter of 0.45- μ m pore size. Discard the first 5 mL. Dilute a suitable volume of the filtrate with Medium, as needed.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 10-cm; 3- μ m packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 μ L

Run time: 2 times the retention time of levetiracetam

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the concentration, C_s , of levetiracetam ($C_8H_{14}N_2O_2$) in Medium (mg/mL) after time point i :

$$\text{Result}_i = (r_u/r_s) \times D \times C_s$$

- r_u = peak response from the Sample solution
 r_s = peak response from the Standard solution
 D = dilution factor, as needed
 C_s = concentration of the Standard solution (mg/mL)

Calculate the percentage of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = C_2 \times V \times (1/L) \times 100 + \text{Result}_1$$

$$\text{Result}_3 = C_3 \times V \times (1/L) \times 100 + \text{Result}_2$$

$$\text{Result}_4 = C_4 \times V \times (1/L) \times 100 + \text{Result}_3$$

C_i = concentration of levetiracetam in the portion of sample withdrawn at the specified time point (mg/mL)

V = volume of Medium, 230 mL

L = label claim (mg/Tablet)

Tolerances: See Table 7.

Table 7

Time Point (i)	Time (h)	Amount Dissolved	
		500 mg/ Tablet (%)	750 mg/ Tablet (%)
1	1	15–35	10–30
2	2	30–50	25–45
3	4	50–75	45–70
	8	NLT 80	—
4	10	—	NLT 80

The percentages of the labeled amount of levetiracetam ($C_8H_{14}N_2O_2$), dissolved at the times specified, conform to Dissolution (711), Acceptance Table 2.

• (RB 1-Apr-2016)

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Solution A: Dilute 2 mL of phosphoric acid with water to 1 L.

Diluent: Acetonitrile and Solution A (5:95)

Buffer: 1.4 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Acetonitrile and Buffer (5:95). To each L of the mixture, add 1 g of sodium 1-hexanesulfonate monohydrate.

System suitability solution: 0.3 mg/mL of USP Levetiracetam RS in Diluent prepared as follows. Dissolve the required amount of USP Levetiracetam RS in 10% of the final volume of 0.1 N potassium hydroxide. Let the mixture react at room temperature for about 15 min, and then neutralize by adding 0.1 N hydrochloric acid at 10% of the flask volume. Dilute with Diluent to volume. [NOTE—This solution contains levetiracetam and levetiracetam acid.]

Standard solution: 12.5 μ g/mL of USP Levetiracetam RS in water. Sonication may be used to aid in dissolution. Pass a portion of the solution through a suitable filter of 0.2- μ m pore size.

Sample solution: Nominally equivalent to 2.5 mg/mL of levetiracetam in water, from a portion of crushed Tablets (NLT 20) prepared as follows. Transfer the weighed amount of crushed Tablet powder to a volumetric flask containing water to fill 80% of final volume. Sonicate in cold water for 10 min. Equilibrate to room temperature. Dilute with water to volume. Pass a portion through a suitable filter of 0.2- μ m pore size.

Alternatively, the Sample solution having a nominal concentration of 2–3 mg/mL of levetiracetam may be prepared as follows. Finely grind NLT 10 Tablets, and transfer an amount equivalent to one Tablet to a suitable volumetric flask. Add NLT 30 mL of acetonitrile. Sonicate for 10 min, and shake using a mechanical shaker for 10 min. Add NLT 30 mL of water, and shake for 15 min using a mechanical shaker. Allow the resulting mixture to equilibrate to room temperature. Add NMT 25% of the final flask volume of acetonitrile. Dilute with water to volume. Centrifuge for 15 min, and pass a portion through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Temperatures

Column: 30°

Autosampler: 10°

Flow rate: 2 mL/min

Injection volume: 20 μ L

Run time: 5 times the retention time of levetiracetam

System suitability

Samples: System suitability solution and Standard solution

Suitability requirements

Resolution: NLT 1.5 between levetiracetam and levetiracetam acid peaks, System suitability solution

Tailing factor: NMT 2.0, Standard solution

Relative standard deviation: NMT 5.0%, Standard solution

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

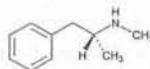
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_S = peak response of USP Levetiracetam RS from the *Standard solution* C_S = concentration of USP Levetiracetam RS in the *Standard solution* (mg/mL) C_U = nominal concentration of levetiracetam in the *Sample solution* (mg/mL)**Acceptance criteria:** See Table 8.**Table 8**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levetiracetam related compound B ^{a,b}	0.40	—
Levetiracetam	1.0	—
Levetiracetam acid ^c	1.3	0.30
Levetiracetam related compound A ^{b,d}	1.9	—
Any individual unspecified degradation product	—	0.10
Total impurities	—	1.0

^a (S)-2-Aminobutanamide.^b Process impurities controlled in the drug substance. Included for identification purposes only. Not reported for the drug product, and not included in total impurities.^c (S)-2-(2-Oxopyrrolidin-1-yl)butanoic acid.^d (S)-N-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- USP REFERENCE STANDARDS (11)**
USP Levetiracetam RS

Levmetamfetamine $C_{10}H_{15}N$ 149.23Benzeneethanamine, N, α -dimethyl-, (R)-.(-)-(R)-N, α -Dimethylphenethylamine [33817-09-3].

» Levmetamfetamine contains not less than 98.0 percent and not more than 100.5 percent of $C_{10}H_{15}N$.

Packaging and storage—Preserve in tight, light-resistant containers.**USP Reference standards (11)**—

USP Levmetamfetamine RS

USP Methamphetamine Hydrochloride RS

Identification—**A:** *Infrared Absorption* (197F).**B:** The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *System suitability solution*, as obtained in the test for *Limit of methamphetamine*.**Specific rotation** (781S): between -18.5° and -21.5° .*Test solution:* 16 mg per mL, in 1.2 N hydrochloric acid.**Limit of methamphetamine—***Mobile phase*—Prepare a filtered and degassed mixture of hexane, isopropyl alcohol, and acetonitrile (98:1.5:0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Resolution solution—Mix suitable quantities of a solution of USP Methamphetamine Hydrochloride RS in chloroform and USP Levmetamfetamine RS in chloroform to obtain a solution containing about 0.025 mg per mL and 2.5 mg per mL of methamphetamine hydrochloride and levmetamfetamine, respectively. Transfer 2.0 mL of this solution to a suitable container, add 10 mg of 2-naphthyl chloroformate and 2.0 mL of chloroform, mix with a vortex mixer, and allow to stand for 5 minutes. To this solution, add 2 mL of 1 N sodium hydroxide, mix with a vortex mixer, allow to stand for 5 minutes, and discard the aqueous layer. Wash the organic layer twice with 2 mL of 1 N sodium hydroxide, discarding the aqueous layer. To the organic layer add 2 mL of 1 N hydrochloric acid, mix with a vortex mixer, and discard the aqueous layer. Wash the organic layer twice with 2 mL of 1 N hydrochloric acid, discarding the aqueous layer. To the organic layer add 2 mL of water, mix with a vortex mixer, and discard the aqueous layer. Wash the organic layer twice with 2 mL of water, discarding the aqueous layer. To the organic layer add about 1.0 g of anhydrous sodium sulfate, and mix with a vortex mixer. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and filter.

Test solution—Transfer about 62.5 mg of Levmetamfetamine, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with chloroform to volume, and mix. Transfer 2.0 mL of this solution to a suitable container, and proceed as directed in *Resolution solution* beginning with "add 10 mg of 2-naphthyl chloroformate and 2 mL of chloroform."

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 274-nm detector and a 4.6-mm \times 25-cm column that contains packing L36. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for methamphetamine and 1.0 for levmetamfetamine; and the resolution, R , between methamphetamine and levmetamfetamine is not less than 1.4. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject a volume (about 50 μ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Calculate the percentage of methamphetamine in the portion of Levmetamfetamine taken by the formula:

$$100r_M / (r_M + r_L)$$

in which r_M is the methamphetamine peak response obtained from the *Test solution*, and r_L is the peak response of levmetamfetamine obtained from the *Test solution*: not more than 0.1% is found.

Limit of nonvolatile residue—Heat about 1.0 g, accurately weighed, at 150° to constant weight: the limit is not more than 0.5%.

Ordinary impurities (466)—

Test solution: chloroform.

Standard solution: chloroform.

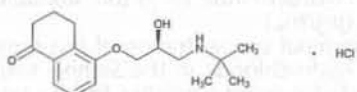
Eluant: a mixture of chloroform, cyclohexane, and diethylamine (5:4:1).

Visualization: 1.

Limits—No impurity exceeds 0.1%, and the total does not exceed 0.5%.

Assay—Transfer about 400 mg of Levmetamfetamine to a suitable container, add 50.0 mL of glacial acetic acid, and mix. Add two drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 14.92 mg of $C_{10}H_{13}N$.

Levobunolol Hydrochloride



$C_{17}H_{25}NO_3 \cdot HCl$ 327.85
 1-(2*H*)-Naphthalenone, 5-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-3,4-dihydro-, hydrochloride, (–)-(S); (–)-5-[3-(*tert*-Butylamino)-2-hydroxypropoxy]-3,4-dihydro-1(2*H*)-naphthalenone hydrochloride [27912-14-7].

DEFINITION

Levobunolol Hydrochloride contains NLT 98.0% and NMT 102.0% of levobunolol hydrochloride ($C_{17}H_{25}NO_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- A. INFRARED ABSORPTION** (197M)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements

ASSAY**PROCEDURE**

Solution A: 5 mM sodium 1-heptanesulfonate in methanol

Solution B: 5 mM sodium 1-heptanesulfonate in water
Mobile phase: *Solution A*, 0.5 M sulfuric acid, and *Solution B* (53:1:47)

Standard solution: 100 µg/mL of USP Levobunolol Hydrochloride RS in *Mobile phase*

Sample solution: 100 µg/mL of Levobunolol Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 223 nm

Column: 3.9 mm × 25 cm; 10-µm packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levobunolol hydrochloride ($C_{17}H_{25}NO_3 \cdot HCl$) in the portion of Levobunolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Levobunolol Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = concentration of Levobunolol Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- RESIDUE ON IGNITION** (281): NMT 0.1%

ORGANIC IMPURITIES

Solution A, Solution B, and Mobile phase: Proceed as directed in the *Assay*.

System suitability solution: 50 µg/mL each of USP Levobunolol Hydrochloride RS and USP Atenolol RS in *Mobile phase*

Standard solution: 5 µg/mL of USP Levobunolol Hydrochloride RS in *Mobile phase*

Sample solution: 1 mg/mL of Levobunolol Hydrochloride in *Mobile phase*

Chromatographic system: Proceed as directed in the *Assay* using *Mobile phase*.

Run time: 3 times the retention time of levobunolol

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 8 between levobunolol and atenolol peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Levobunolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of levobunolol from the *Standard solution*

C_S = concentration of USP Levobunolol Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = concentration of Levobunolol Hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria

Individual impurity: NMT 0.5%

Total impurities: NMT 1%

SPECIFIC TESTS

- OPTICAL ROTATION, Specific Rotation** (781S)

Sample solution: 30 mg/mL in methanol

Acceptance criteria: -19° to -20°

- pH** (791)

Sample: 50 mg/mL

Acceptance criteria: 4.5–6.5

- LOSS ON DRYING** (731)

Sample: 1 g

Analysis: Dry under vacuum over phosphorus pentoxide at 110° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers. Protect from light. Store at room temperature.

- USP REFERENCE STANDARDS** (11)

USP Atenolol RS

USP Levobunolol Hydrochloride RS

Levobunolol Hydrochloride Ophthalmic Solution

DEFINITION

Levobunolol Hydrochloride Ophthalmic Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of levobunolol hydrochloride ($C_{17}H_{25}NO_3 \cdot HCl$).

IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Solution A: 5 mM sodium 1-heptanesulfonate in water
Mobile phase: Methanol, glacial acetic acid, and *Solution A* (550:5:450)

Standard solution: 50 µg/mL of USP Levobunolol Hydrochloride RS in *Mobile phase*

Sample solution: Nominally equivalent to 50 µg/mL of levobunolol hydrochloride in *Mobile phase* from Ophthalmic Solution

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9 mm × 30 cm; 10-µm packing L1

Flow rate: 1.5 mL

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.2

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levobunolol hydrochloride ($C_{17}H_{25}NO_3 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Levobunolol Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levobunolol hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

ORGANIC IMPURITIES

Mobile phase: Proceed as directed in the *Assay*.

Standard solution: 10 µg/mL each of USP Levobunolol Hydrochloride RS and USP Edetate Disodium RS in *Mobile phase*

Sample solution: Nominally equivalent to 1 mg/mL of levobunolol hydrochloride in *Mobile phase* from Ophthalmic Solution

Chromatographic system and System suitability: Proceed as directed in the *Assay* using wavelengths of UV 254 nm and 400 nm.

[NOTE—The relative retention times for levobunolol and edetate disodium are 1.0 and 0.46, respectively.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity at 254 nm in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of levobunolol from the *Standard solution*

C_S = concentration of USP Levobunolol Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of levobunolol hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of any impurity at the retention time of levobunolol (obtained using the detector at 254 nm) using the detector at 400 nm in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the impurity from the *Sample solution* at 400 nm

r_S = peak response of levobunolol from the *Standard solution* at 254 nm

C_S = concentration of USP Levobunolol Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of levobunolol hydrochloride in the *Sample solution* (µg/mL)

F = relative response factor for the impurity, 0.2

Acceptance criteria

Individual impurity: NMT 1%

Total impurities: NMT 2.5%. Disregard any peak obtained with the detector at 254 nm with the retention time of edetate disodium.

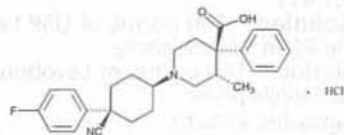
SPECIFIC TESTS

- ANTIMICROBIAL EFFECTIVENESS TESTING (51):** Meets the requirements
- STERILITY TESTS (71):** Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- PH (791):** 5.5–7.5

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers. Protect from light. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
USP Edetate Disodium RS
USP Levobunolol Hydrochloride RS

Levocabastine Hydrochloride



$C_{26}H_{29}FN_2O_2 \cdot HCl$ 456.98
4-Piperidinecarboxylic acid, 1-[4-cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenyl-, monohydrochloride, (–)-[1(*cis*), 3 α , 4 β]-;
(–)-*trans*-1-[*cis*-4-Cyano-4-(*p*-fluorophenyl)cyclohexyl]-3-methyl-4-phenylisopropionic acid monohydrochloride [79547-78-7].

DEFINITION

Levocabastine Hydrochloride contains NLT 98.5% and NMT 101.5% of $C_{26}H_{29}FN_2O_2 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL**, Chloride (191): Meets the requirements
- **C. OPTICAL ROTATION**, Specific Rotation (781S): Meets the requirements

ASSAY• **PROCEDURE**

Sample solution: Dissolve 175 mg of Levocabastine Hydrochloride in 50 mL of alcohol, and add 5.0 mL of 0.01 N hydrochloric acid.

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N sodium hydroxide VS

Endpoint detection: Potentiometric

Analysis

Sample: *Sample solution*

The volume of titrant required to titrate Levocabastine Hydrochloride is the difference between the first and third endpoints. Perform a blank determination and make any necessary correction. Each mL of 0.1 N sodium hydroxide VS is equivalent to 22.85 mg of $C_{26}H_{29}FN_2O_2 \cdot HCl$.

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES**Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%, based on a sample weight of about 1.000 g

Organic Impurities• **PROCEDURE**

[NOTE—Prepare solutions immediately before use.]

Diluent: 2 mg/mL of sodium hydroxide in water

Solution A: Dissolve 1.39 g of boric acid in water, and adjust with 1 N sodium hydroxide to a pH of 9.0. Dilute with water to 100 mL.

Run buffer: Dissolve 1.08 g of sodium dodecyl sulfate and 650 mg of hydroxypropyl- β -cyclodextrin in 5 mL of isopropyl alcohol, then dilute with *Solution A* to 50 mL.

System suitability solution: 12.5 μ g/mL of USP Levocabastine Hydrochloride RS and 12.5 μ g/mL of USP Levocabastine Related Compound A RS in *Diluent*

Standard solution: Dilute 5.0 mL of the *Sample solution* with *Diluent* to 100 mL. Dilute 1.0 mL of this solution with *Diluent* to 10 mL to obtain a solution containing 12.5 μ g/mL of Levocabastine Hydrochloride.

Sample solution: 2.5 mg/mL of Levocabastine Hydrochloride in *Diluent*

Capillary electrophoresis system

Detector: UV 214 nm

Column: 75- μ m \times 50-cm uncoated fused-silica capillary column

Column temperature: 50°

Current: See the gradient table below.

Time (min)	Current (μ A)
0	0
0.17	75
15	130
40	130
60	200

[NOTE—Before performing the *System suitability*, equilibrate the capillary column with *Diluent* for 2 min, then equilibrate with *Run buffer* for at least 5 min.]

System suitability

Sample: *System suitability solution*

[NOTE—The relative migration times for levocabastine and levocabastine related compound A are approximately 1.0 and 1.07, respectively.]

Suitability requirements

Resolution: NLT 4 between levocabastine and levocabastine related compound A

[NOTE—If necessary, adjust the current gradient to achieve the required resolution.]

Analysis

Samples: *Diluent* (blank), *Standard solution*, and *Sample solution*

Separately inject equal volumes (pressure of 3450 Pa for 5 s) of the *Samples*, and record the peak responses.

[NOTE—Disregard any peak originating from the *Diluent*. Disregard any peak with an area of less than 0.1 times the major peak area of the *Standard solution* (0.05%).]

Acceptance criteria: The area for any peak in the *Sample solution*, other than the major peak, is not greater than the major peak area of the *Standard solution* (0.5%); and the sum of all peak areas in the *Sample solution*, except for the major peak, is not greater than twice the major peak area of the *Standard solution* (1.0%).

SPECIFIC TESTS

- **OPTICAL ROTATION**, Specific Rotation (781S): -102° to -106° at 20°

Sample solution: 10 mg/mL in methanol

- **LOSS ON DRYING** (731): Dry about 1.000 g of the sample at 105° to constant weight: it loses NMT 0.5% of its weight.

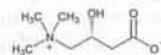
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Protect from light.

- **USP REFERENCE STANDARDS** (11)

USP Levocabastine Hydrochloride RS

USP Levocabastine Related Compound A RS

Levocarnitine

$C_7H_{15}NO_3$ 161.20
(*R*)-3-Carboxy-2-hydroxy-*N,N,N*-trimethyl-1-propanaminium, inner salt;
(*R*)-(3-Carboxy-2-hydroxypropyl)trimethylammonium, inner salt [541-15-1].

DEFINITION

Levocarnitine contains NLT 97.0% and NMT 103.0% of levocabastine ($C_7H_{15}NO_3$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

Analysis: Dry the sample and the USP Levocabastine RS under vacuum at 50° for 5 h.

Acceptance criteria: Meets the requirements

ASSAY• **PROCEDURE**

Sample: 100 mg of Levocabastine

Blank: A mixture of 3 mL of formic acid and 50 mL of glacial acetic acid

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid. Add

2 drops of crystal violet TS, and titrate with the *Titrant* to an emerald green endpoint. Perform the *Blank* determination.

Calculate the percentage of levocarnitine ($C_7H_{15}NO_3$) in the portion of Levocarnitine taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F] / W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 161.2 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.5%

• **CHLORIDE AND SULFATE**, *Chloride* (221)

Standard: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.090 g of Levocarnitine

Acceptance criteria: NMT 0.4%

Delete the following:

• **HEAVY METALS** (231): NMT 20 ppm (Official 1-Jan-2018)

• **LIMIT OF POTASSIUM**

[NOTE—The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

Standard solution: 31.25 µg/mL of potassium in water, prepared from potassium chloride, previously dried at 105° for 2 h

Sample stock solution: 0.625 mg/mL of Levocarnitine in water

Sample solution A: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with water to volume. This solution contains 500 µg/mL of Levocarnitine and 0 µg/mL of added potassium from the *Standard solution*.

Sample solution B: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 2.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 500 µg/mL of Levocarnitine and 2.5 µg/mL of added potassium from the *Standard solution*.

Sample solution C: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 4.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 500 µg/mL of Levocarnitine and 5.0 µg/mL of added potassium from the *Standard solution*.

Blank: Water

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 766.7 nm

Lamp: Potassium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Sample solution A*, *Sample solution B*, *Sample solution C*, and *Blank*

Determine the absorbances of the solutions against the *Blank*. Plot the absorbances of the three *Sample solutions* versus their added potassium concentrations, in µg/mL. Draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the

concentration, in µg/mL, of potassium in *Sample solution A*.

Calculate the percentage of potassium in the portion of Levocarnitine taken:

$$\text{Result} = (C_K / C_U) \times 100$$

C_K = concentration of potassium in *Sample solution A* (µg/mL), determined from the intercept of the linear regression line

C_U = concentration of Levocarnitine in *Sample solution A* (µg/mL)

Acceptance criteria: NMT 0.2%

• LIMIT OF SODIUM

[NOTE—The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

Standard solution: 250 µg/mL of sodium in water, prepared from sodium chloride, previously dried at 105° for 2 h

Sample stock solution: 40.0 mg/mL of Levocarnitine in water

Sample solution A: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with water to volume. This solution contains 32 mg/mL of Levocarnitine and 0 µg/mL of added sodium from the *Standard solution*.

Sample solution B: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 2.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 32 mg/mL of Levocarnitine and 20 µg/mL of added sodium from the *Standard solution*.

Sample solution C: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 4.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 32 mg/mL of Levocarnitine and 40 µg/mL of added sodium from the *Standard solution*.

Blank: Water

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 589.0 nm

Lamp: Sodium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Sample solution A*, *Sample solution B*, *Sample solution C*, and *Blank*

Determine the absorbances of the solutions against the *Blank*. Plot the absorbances of the three *Sample solutions* versus their added sodium concentrations, in µg/mL. Draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the concentration, in µg/mL, of sodium in *Sample solution A*.

Calculate the percentage of sodium in the portion of Levocarnitine taken:

$$\text{Result} = (C_{Na} / C_U) \times 100$$

C_{Na} = concentration of sodium in *Sample solution A* (µg/mL), determined from the intercept of the linear regression line

C_U = concentration of Levocarnitine in *Sample solution A* (µg/mL)

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation (781S)**
Sample solution: 100 mg/mL in water
Acceptance criteria: -29° to -32°
- **pH (791)**
Sample solution: 50 mg/mL solution
Acceptance criteria: 5.5–9.5
- **WATER DETERMINATION (921):** NMT 4.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Levocarnitine RS

Levocarnitine Injection

DEFINITION

Levocarnitine Injection is a sterile solution of Levocarnitine in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of levocarnitine ($C_7H_{15}NO_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B. COLOR REACTION**
Analysis: Transfer 2 mL of Injection to a test tube, add 5 mL of 1 N hydrochloric acid and a few drops of ammonium reineckate TS.
Acceptance criteria: A red-violet precipitate is produced.

ASSAY

• PROCEDURE

Buffer: 0.05 M phosphate buffer, prepared by dissolving 6.805 g of monobasic potassium phosphate in 1 L of water

Mobile phase: Acetonitrile and *Buffer* (65:35). Adjust with phosphoric acid to a pH of 4.7, and mix.

System suitability solution: 5 mg/mL of USP Levocarnitine RS and 0.024 mg/mL of USP Levocarnitine Related Compound A RS in water

Standard solution: 10 mg/mL of USP Levocarnitine RS in water

Sample solution: Pool the contents of 10 containers, and dilute an accurately measured volume of Injection quantitatively with water to obtain a solution having a nominal concentration of about 10 mg/mL of levocarnitine.

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L8

Flow rate: 1 mL/min

Injection volume: 5 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between levocarnitine related compound A (crotonylbetaine) and levocarnitine, *System suitability solution*

Relative standard deviation: NMT 2.0% for levocarnitine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levocarnitine ($C_7H_{15}NO_3$) in the portion of Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak area of levocarnitine from the *Sample solution*

r_s = peak area of levocarnitine from the *Standard solution*

C_s = concentration of USP Levocarnitine RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of levocarnitine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.1 USP Endotoxin Units/mg of levocarnitine.
- **pH (791):** 6.0–6.5
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS (1):** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass. Store below 25°. Do not freeze.
- **USP REFERENCE STANDARDS (11)**
USP Endotoxin RS
USP Levocarnitine RS
USP Levocarnitine Related Compound A RS
2-Propen-1-aminium, 3-carboxy-*N,N,N*-trimethyl-, chloride.
 $C_7H_{14}ClNO_2$ 179.65

Levocarnitine Oral Solution

DEFINITION

Levocarnitine Oral Solution is a solution of levocarnitine in water, and it contains suitable antimicrobial agents. It may contain a suitable flavor. It contains NLT 90.0% and NMT 110.0% of the labeled amount of levocarnitine ($C_7H_{15}NO_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the Assay.

ASSAY

• PROCEDURE

Buffer: 0.05 M phosphate buffer, pH 2.4, prepared by mixing 11.5 mL of phosphoric acid, 1900 mL of water, and about 100 mL of 1 N sodium hydroxide

Mobile phase: Dissolve 555 mg of sodium 1-heptanesulfonate in 980 mL of *Buffer* with stirring. Add 20 mL of methanol, and mix.

Internal standard solution: 0.02 mg/mL of *p*-aminobenzoic acid in water

Standard solution: Transfer about 10 mg of USP Levocarnitine RS to a 5-mL volumetric flask, add 1.0 mL of the *Internal standard solution*, and dilute with water to volume.

Sample stock solution: Equivalent to 10 mg/mL of levocarnitine in water from an accurately measured volume of Oral Solution

Sample solution: Wash a 10-mm \times 4-cm disposable column containing 500 mg of packing L1, in order,

with two column volumes of methylene chloride, two column volumes of methanol, and three column volumes of water. Pipet 5.0 mL of the *Sample stock solution* into the washed disposable column, and rinse the column twice with 6.0-mL portions of water. Collect the filtrate and washings in a 25-mL volumetric flask, add 5.0 mL of the *Internal standard solution*, and dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 3.9-mm × 30-cm; 10-μm packing L1

Flow rate: 1.5 mL/min

Injection size: 40 μL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for levocarnitine and *p*-aminobenzoic acid are 0.56 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between the levocarnitine and internal standard peaks

Relative standard deviation: NMT 2.0% for levocarnitine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levocarnitine ($C_7H_{15}NO_3$) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of levocarnitine to *p*-aminobenzoic acid from the *Sample solution*

R_S = peak area ratio of levocarnitine to *p*-aminobenzoic acid from the *Standard solution*

C_S = concentration of USP Levocarnitine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levocarnitine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **PH** (791): 4.0–6.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)
USP Levocarnitine RS

Levocarnitine Tablets

DEFINITION

Levocarnitine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of levocarnitine ($C_7H_{15}NO_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

- **B. COLOR REACTION**

Analysis: Dissolve 1 Tablet in 5 mL of water, filter, and add 5 mL of 1 N hydrochloric acid. Place 2 mL of the filtrate in a test tube, and add a few drops of ammonium reineckate TS.

Acceptance criteria: A red-violet precipitate is produced.

ASSAY

• PROCEDURE

Buffer: 0.05 M phosphate buffer, pH 4.5, prepared by dissolving 6.805 g of monobasic potassium phosphate in 1 L of water

Mobile phase: Acetonitrile and *Buffer* (65:35). Adjust with phosphoric acid to a pH of 4.7, and mix.

System suitability solution: 1.5 mg/mL of USP Levocarnitine RS and 7 μg/mL of USP Levocarnitine Related Compound A RS in water

Standard solution: 3 mg/mL of USP Levocarnitine RS in water

Sample solution: Transfer 10 Tablets, accurately weighed, to a 500-mL volumetric flask, and add water to volume. Shake until the Tablets have disintegrated completely, and pass through a filter of 0.45-μm pore size. Dilute a portion of the filtrate quantitatively with water to a nominal concentration of about 3 mg/mL of levocarnitine.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 3.9-mm × 30-cm; 10-μm packing L8

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between levocarnitine related compound A (crotonoylbetaine) and levocarnitine, *System suitability solution*

Relative standard deviation: NMT 2.0% for levocarnitine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levocarnitine ($C_7H_{15}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of levocarnitine from the *Sample solution*

r_S = peak area of levocarnitine from the *Standard solution*

C_S = concentration of USP Levocarnitine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levocarnitine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** (711)

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 30 min

Standard solution: Known concentration of USP Levocarnitine RS in *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium* if necessary

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the Assay, making any necessary modifications.

Determine the percentage of the labeled amount of levocarnitine ($C_7H_{15}NO_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

r_U = peak area of levocarnitine in the *Sample solution*

r_S = peak area of levocarnitine in the *Standard solution*

- C_s = concentration of USP Levocarnitine RS in the *Standard solution* (mg/mL)
 D = dilution factor for the *Sample solution*
 V = volume of *Medium*, 900 mL
 L = label claim (mg/Tablet)

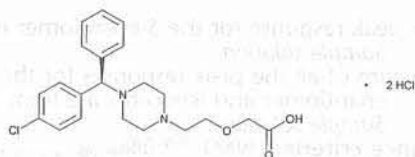
Tolerances: NLT 75% (Q) of the labeled amount of levocarnitine ($C_7H_{15}NO_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Weight Variation*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
 USP Levocarnitine RS
 USP Levocarnitine Related Compound A RS
 2-Propen-1-aminium, 3-carboxy-*N,N,N*-trimethyl-, chloride.
 $C_7H_{14}ClNO_2$ 179.65

Levocetirizine Dihydrochloride



$C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ 461.81

Acetic acid, [2-[4-[(*R*)-(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-, dihydrochloride;
 (2-[4-[(*R*)-(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy)acetic acid dihydrochloride [130018-87-0].
 Levocetirizine free base

$C_{21}H_{25}ClN_2O_3$ 388.89
 [130018-77-8].

DEFINITION

Levocetirizine Dihydrochloride contains NLT 98.0% and NMT 102.0% of levocetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the levocetirizine peak of the *System suitability solution*, as obtained in the test for *Enantiomeric Purity*.
- **C. IDENTIFICATION TESTS—GENERAL (191), Chloride:** Meets the requirements

ASSAY

PROCEDURE

Mobile phase: Acetonitrile, water, and 1 M sulfuric acid (93: 6.6: 0.4)

Standard solution: 0.05 mg/mL of USP Levocetirizine Dihydrochloride RS in *Mobile phase*

Sample solution: 0.05 mg/mL of Levocetirizine Dihydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L3

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levocetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) in the portion of Levocetirizine Dihydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of levocetirizine from the *Sample solution*

r_s = peak response of levocetirizine from the *Standard solution*

C_s = concentration of USP Levocetirizine Dihydrochloride RS in the *Standard solution* (mg/mL)

C_u = concentration of Levocetirizine Dihydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Change to read:

- **RESIDUE ON IGNITION (281):** NMT 0.2% (RB 1-Apr-2016)

Change to read:

ORGANIC IMPURITIES

Mobile phase: Acetonitrile, water, and 1 M sulfuric acid (93: 6.6: 0.4)

System suitability solution: 0.2 mg/mL of USP Levocetirizine Dihydrochloride RS and 0.2 μ g/mL each of USP Levocetirizine Amide RS and USP Chlorobenzhydryl Piperazine RS in *Mobile phase*. Use the solution within 16 h.

Standard solution: 0.2 μ g/mL each of USP Levocetirizine Dihydrochloride RS, USP Levocetirizine Amide RS, and USP Chlorobenzhydryl Piperazine RS in *Mobile phase*. Use the solution within 16 h.

Sample solution: 200 μ g/mL of Levocetirizine Dihydrochloride in *Mobile phase*. Use the solution within 16 h.

Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L3

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: 3 times the retention time of levocetirizine

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between levocetirizine and chlorobenzhydryl piperazine, *System suitability solution*

Tailing factor: NMT 2.0 for levocetirizine, *System suitability solution*

Relative standard deviation: NMT 5.0% for levocetirizine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levocetirizine amide or chlorobenzhydryl piperazine in the portion of Levocetirizine Dihydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levocetirizine amide or chlorobenzhydryl piperazine from the *Sample solution*

r_S = peak response of levocetirizine amide or chlorobenzhydryl piperazine from the *Standard solution*

C_S = concentration of USP Levocetirizine Amide RS or USP Chlorobenzhydryl Piperazine RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Levocetirizine Dihydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

Calculate the percentage of any unspecified impurity in the portion of Levocetirizine Dihydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_S = peak response of levocetirizine from the *Standard solution*

C_S = concentration of USP Levocetirizine Dihydrochloride RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Levocetirizine Dihydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levocetirizine dihydrochloride	1.0	—
Chlorobenzhydryl piperazine	1.3	0.2% (RB 1-Apr-2016)
Levocetirizine amide	2.5	0.2% (RB 1-Apr-2016)
Any individual unspecified impurity	—	0.1% (RB 1-Apr-2016)
Total impurities	—	0.5% (RB 1-Apr-2016)

Change to read:

• **ENANTIOMERIC PURITY**

Analyze the solutions within 24 h of preparation.

Mobile phase: Prepare a mixture of chromatographic solvent hexane and absolute alcohol (95:5). Add 2 mL of trifluoroacetic acid per L of mixture.

System suitability solution: 5 mg/mL of USP Cetirizine Hydrochloride RS in absolute alcohol

Sensitivity solution: 0.05 mg/mL of USP Levocetirizine Dihydrochloride RS in absolute alcohol

Sample solution: 5 mg/mL of Levocetirizine Dihydrochloride in absolute alcohol

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L40

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—The relative retention times for the S-enantiomer (of cetirizine) and levocetirizine, which is the R-enantiomer of cetirizine, are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between the S-enantiomer and levocetirizine, *System suitability solution*

Signal-to-noise ratio: NLT 10 for levocetirizine, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of the S-enantiomer in the portion of Levocetirizine Dihydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for the S-enantiomer from the *Sample solution*

r_T = sum of all the peak responses for the S-enantiomer and levocetirizine from the *Sample solution*

Acceptance criteria: NMT 2.0% (RB 1-Apr-2016) of the S-enantiomer

SPECIFIC TESTS**Change to read:**

• **LOSS ON DRYING** (731)

Analysis: Dry at 105° to constant weight.

Acceptance criteria: NMT 1.0% (RB 1-Apr-2016)

• **PH** (791)

Sample solution: 50 mg/mL of Levocetirizine Dihydrochloride in water

Acceptance criteria: 1.2–1.8

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Cetirizine Hydrochloride RS

USP Chlorobenzhydryl Piperazine RS

(R)-1-[(4-Chlorophenyl)phenylmethyl]piperazine.

$\text{C}_{17}\text{H}_{19}\text{ClN}_2$ 286.80

USP Levocetirizine Amide RS

(R)-2-(2-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy)acetamide.

$\text{C}_{21}\text{H}_{26}\text{ClN}_3\text{O}_2$ 387.90

USP Levocetirizine Dihydrochloride RS

Levocetirizine Dihydrochloride Tablets**DEFINITION**

Levocetirizine Dihydrochloride Tablets contain NLT 90% and NMT 110% of the labeled amount of levocetirizine dihydrochloride ($\text{C}_{21}\text{H}_{25}\text{ClN}_2\text{O}_3 \cdot 2\text{HCl}$).

IDENTIFICATION

• **A. ULTRAVIOLET ABSORPTION** (197U)

Medium: Water

Sample solution: Nominally 0.1 mg/mL of levocetirizine dihydrochloride from Tablets in water prepared as follows. Transfer 1 Tablet to a suitable volumetric

flask, and add 40% of the flask volume of water. Shake for NLT 5 min to promote the disintegration of the Tablet. Dilute with water to volume. Pass a 10-mL portion of the resulting solution through a suitable filter, and discard the first mL. Use the filtrate.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Solution A: 1 M sulfuric acid and water (5.7: 94.3)

Mobile phase: Acetonitrile, water, and 1 M sulfuric acid (93: 6.6: 0.4)

System suitability solution: 0.2 mg/mL of USP Levocetirizine Dihydrochloride RS and 0.2 µg/mL of USP Chlorobenzhydryl Piperazine RS in *Mobile phase*

Standard solution: 0.2 mg/mL of USP Levocetirizine Dihydrochloride RS in *Mobile phase*

Sample solution: Nominally 0.2 mg/mL of levocetirizine dihydrochloride prepared as follows. Transfer a number of Tablets (NLT 10), equivalent to 50 mg of levocetirizine dihydrochloride, to a 250-mL volumetric flask. Add 20 mL of *Solution A*, and put the flask on a mechanical shaker for 15 min. Add 150 mL of acetonitrile, and place the flask in an ultrasonic bath for 10 min. Allow the contents to cool to room temperature, if necessary, and dilute with acetonitrile to final volume. Homogenize the solution, and centrifuge a 10-mL portion for 5 min. Use the supernatant solution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Columns

Guard: 4-mm × 0.3-cm; 5-µm packing L3

Analytical: 4.6-mm × 25-cm; 5-µm packing L3

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between levocetirizine and chlorobenzhydryl piperazine

Tailing factor: NMT 1.5 for levocetirizine

Relative standard deviation: NMT 1.0% for levocetirizine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levocetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levocetirizine from the *Sample solution*

r_S = peak response of levocetirizine from the *Standard solution*

C_S = concentration of USP Levocetirizine Dihydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levocetirizine dihydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90%–110%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: (L/900) mg/mL of USP Levocetirizine Dihydrochloride RS in *Medium*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV-Vis

Analytical wavelength: 230 or 231 nm; use a suitable wavelength for background correction

Cell: 1 or 2 cm

Blank: *Medium*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of the labeled amount of levocetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Levocetirizine Dihydrochloride RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of levocetirizine dihydrochloride ($C_{21}H_{25}ClN_2O_3 \cdot 2HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

ORGANIC IMPURITIES

Solution A, Mobile phase, System suitability solution, and Sample solution: Prepare as directed in the *Assay*.

Standard solution: 0.002 mg/mL of USP Levocetirizine Dihydrochloride RS in *Mobile phase*

Chromatographic system: Proceed as directed in the *Assay*, except for the *Run time*.

Run time: 2.3 times the retention time of levocetirizine

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between levocetirizine and chlorobenzhydryl piperazine

Tailing factor: NMT 1.5 for levocetirizine

Relative standard deviation: NMT 1.0% for levocetirizine; NMT 5.0% for chlorobenzhydryl piperazine

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of levocetirizine from the *Standard solution*

C_S = concentration of USP Levocetirizine Dihydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levocetirizine dihydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of levocetirizine (free base), 388.89

M_{r2} = molecular weight of levocetirizine dihydrochloride, 461.81

Acceptance criteria: See Table 1. Disregard peaks less than 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levocetirizine	1.0	—
Chlorobenzhydryl piperazine ^a	1.4	—
Levocetirizine amide ^{a,b}	2.1	—
Any individual unspecified degradation product	—	0.30
Total impurities	—	1.0

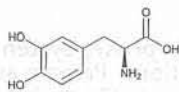
^a This is a process impurity that is included in this table for identification only. This impurity is controlled in the drug substance. This impurity is not to be reported for the drug product and is not to be included in the total impurities.

^b (R)-2-(2-[(4-(4-Chlorophenyl)phenylmethyl)piperazin-1-yl]ethoxy)-acetamide.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Chlorobenzhydryl Piperazine RS
(R)-1-[(4-Chlorophenyl)phenylmethyl]piperazine.
 $C_{17}H_{19}ClN_2$ 286.80
USP Levocetirizine Dihydrochloride RS

Levodopa



$C_9H_{11}NO_4$ 197.19
L-Tyrosine, 3-hydroxy-;
(-)-3-(3,4-Dihydroxyphenyl)-L-alanine [59-92-7].

DEFINITION

Levodopa contains NLT 98.0% and NMT 102.0% of levodopa ($C_9H_{11}NO_4$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Protect all solutions from light, and maintain them at 10° until they are injected into the chromatograph.

Diluent: 0.1% trifluoroacetic acid in water

Mobile phase: Tetrahydrofuran and *Diluent* (3:97)

System suitability solution: 10 µg/mL each of USP Levodopa RS, USP Levodopa Related Compound B RS, and USP L-Tyrosine RS in *Diluent*

Standard solution: 0.4 mg/mL of USP Levodopa RS in *Diluent*

Sample solution: 0.4 mg/mL of Levodopa in *Diluent*

Chromatographic system
(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm L1 packing

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—Refer to Table 1 in the test for *Organic Impurities* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between levodopa and L-tyrosine

Tailing factor: NMT 2.0 for levodopa

Relative standard deviation: NMT 2.0 for levodopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levodopa ($C_9H_{11}NO_4$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*

r_S = peak response of the *Standard solution*

C_S = concentration of USP Levodopa RS in the *Standard solution* (mg/mL)

C_U = concentration of Levodopa in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1, Jan-2018)

ORGANIC IMPURITIES

Protect all solutions from light, and maintain them at 10° until they are injected into the chromatograph.

Diluent, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Levodopa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area for any impurity in the *Sample solution*

r_S = peak area for Levodopa in the *Standard solution*

C_S = concentration of USP Levodopa RS in the *Standard solution* (mg/mL)

C_U = concentration of Levodopa in the *Sample solution* (mg/mL)

F = relative response factor of each impurity (see Table 1)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levodopa related compound A ^a	0.9	0.41	0.1
Levodopa	1.0	—	—
L-Tyrosine	1.3	0.44	0.1

^a 3-(3,4,6-Trihydroxyphenyl)alanine.

^b 3-(3,4-Dimethoxyphenyl)-L-alanine.

Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levodopa related compound B	1.6	0.83	0.5
L-Veratrylglycine ^b	2.7	0.76	0.1
Individual unknown impurity	—	1.0	0.1
Total impurities	—	—	1.1

^a 3-(3,4,6-Trihydroxyphenyl)alanine.^b 3-(3,4-Dimethoxyphenyl)-L-alanine.**SPECIFIC TESTS**• **OPTICAL ROTATION, Specific Rotation (781S)**

Sample solution: 500 mg of Levodopa in a 25-mL volumetric flask. Add 10 mL of 1 N hydrochloric acid to dissolve the solid, add 5 g of methenamine, swirl the contents to dissolve the methenamine, and add 1 N hydrochloric acid to volume.

Analysis: Allow the *Sample solution* to stand in the dark at 25° for 3 h, and measure the rotation.

Acceptance criteria: −160° to −167°

• **LOSS ON DRYING (731)**

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, store in a dry place, and prevent exposure to excessive heat.

• **USP REFERENCE STANDARDS (11)**

USP Levodopa RS

USP Levodopa Related Compound B RS

3-Methoxytyrosine.

C₁₀H₁₃NO₄ 211.22

USP L-Tyrosine RS

Levodopa Capsules**DEFINITION**

Levodopa Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of levodopa (C₉H₁₁NO₄).

IDENTIFICATION• **A. INFRARED ABSORPTION (197M)**

Sample: Shake a quantity of the contents of the Capsules, equivalent to about 500 mg of levodopa, with 25 mL of 3 N hydrochloric acid, and filter. Adjust the acidity of the filtrate with 6 N ammonium hydroxide to a pH of 3, added dropwise with stirring, and allow to stand protected from light for several h. Filter, wash the precipitate with water, and dry at 105°.

Acceptance criteria: The residue meets the requirements.

ASSAY• **PROCEDURE**

Standard solution: 35 µg/mL of USP Levodopa RS in 0.1 N hydrochloric acid

Sample stock solution: 1.75 mg/mL of levodopa in 0.1 N hydrochloric acid, from the contents of NLT 20 Capsules. Shake the mixture by mechanical means for 5 min, and filter discarding the first 20 mL of the filtrate.

Sample solution: 35 µg/mL of levodopa in 0.1 N hydrochloric acid from the *Sample stock solution*

Blank: 0.1 N hydrochloric acid

Instrumental conditions

Mode: UV-Vis

Cell: 1 cm

Analytical wavelength: 280 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levodopa (C₉H₁₁NO₄) in the portion of Capsule contents taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Levodopa RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of levodopa in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 280 nm

Standard solution: USP Levodopa RS in *Medium*

Sample solution: *Sample per Dissolution (711)*. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of levodopa (C₉H₁₁NO₄) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES• **ORGANIC IMPURITIES**

[NOTE—Use low-actinic glassware for all volumetric solutions.]

Diluent: Dissolve 100 mg of sodium metabisulfite in 10 mL of 1.2 N hydrochloric acid, and dilute with acetone to 100 mL.

Standard solution A: 10 µg/mL of USP Levodopa Related Compound A RS and 10 mg/mL of USP Levodopa RS in *Diluent*

Standard solution B: 50 µg/mL of USP Levodopa Related Compound B RS in *Diluent*

Sample solution: Just prior to application, dissolve 100 mg of the residue obtained in the *Identification* test in 10.0 mL of *Diluent*.

Chromatographic system

(See *Chromatography (621)*, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: Thin-layer chromatographic plate, coated with a 0.25-mm layer of microcrystalline cellulose
Predevelop a plate in *Developing solvent* until the solvent front has moved NLT 18 cm from the origin.
 Remove the plate from the chamber, and dry in a current of air for about 10 min.

[NOTE—The plate may be developed overnight: solvent overflow during predevelopment is of no consequence.]

Application volume: 10 µL

Developing solvent: Butyl alcohol, methanol, glacial acetic acid, and water (150:15:75:75)

Spray reagent: Just before use, mix 2 volumes of ferric chloride solution (100 mg/mL) with 1 volume of potassium ferricyanide solution (50 mg/mL) to obtain about 100 mL of solution.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* at separate points about 3 cm from the bottom of the plate. Dry the spots in a stream of nitrogen, and develop the chromatogram in a suitable low-actinic chamber equilibrated for 5 min with a freshly mixed portion of *Developing solvent* until the solvent front has moved about 15 cm from the line of application. Remove the plate from the chamber, mark the solvent front, and dry in a current of air for about 10 min. Spray the plate with *Spray reagent*. Levodopa related compound A produces a spot at an R_f of about 0.25, and levodopa related compound B at an R_f of about 0.5.

Acceptance criteria: No spot at R_f 0.25 from the *Sample solution* is greater in size or intensity than the corresponding spot from *Standard solution A* corresponding to NMT 0.1% levodopa related compound A. No spot at R_f 0.5 from the *Sample solution* is greater in size or intensity than the corresponding spot from *Standard solution B* corresponding to NMT 0.5% of levodopa related compound B. [NOTE—Disregard the bleached spot, which is an artifact resulting from the development of sodium metabisulfate from *Diluent*. It may appear at an R_f value of about 0.6.]

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, in a dry place, and prevent exposure to excessive heat.
- **USP REFERENCE STANDARDS (11)**
 - USP Levodopa RS
 - USP Levodopa Related Compound A RS
3-(3,4,6-Trihydroxyphenyl)alanine.
 $C_9H_{11}NO_5$ 213.19
 - USP Levodopa Related Compound B RS
3-Methoxytyrosine.
 $C_{10}H_{13}NO_4$ 211.22

Levodopa Tablets**DEFINITION**

Levodopa Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of levodopa ($C_9H_{11}NO_4$).

IDENTIFICATION• **A. INFRARED ABSORPTION (197M)**

Sample: Shake a quantity of powdered Tablets equivalent to 500 mg of levodopa with 25 mL of 3 N hydrochloric acid, and filter. Adjust the acidity of the filtrate with 6 N ammonium hydroxide, added dropwise with stirring, and allow to stand, protected from light, for several h. Filter, wash the precipitate with water, and dry at 105°.

Acceptance criteria: The residue meets the requirements.

ASSAY• **PROCEDURE**

Protect all solutions from light, and maintain them at 10° until they are injected into the chromatograph.

Mobile phase: 0.01 M monobasic potassium phosphate; adjust with phosphoric acid and acetonitrile (97:3) to a pH of 2.0.

Standard solution: 0.4 mg/mL of USP Levodopa RS in *Mobile phase*

Sample solution: 0.4 mg/mL of levodopa in *Mobile phase*, from finely powdered Tablets (NLT 20). Filter. Sonicate for 5 min.

System suitability solution: 10 µg/mL each of USP Levodopa RS, USP Levodopa Related Compound B RS,

and USP Levodopa Related Compound A RS in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.0-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for levodopa related compound A, levodopa, and levodopa related compound B are 0.7, 1.0, and 2.8, respectively.]

Suitability requirements

Resolution: NLT 3.5 between levodopa related compound A and levodopa

Relative standard deviation: NMT 2.0% for levodopa

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levodopa ($C_9H_{11}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Levodopa RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levodopa in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Detector: UV maximum at about 280 nm

Standard solution: USP Levodopa RS in *Medium*

Sample solution: Sample per *Dissolution* (711). Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of levodopa ($C_9H_{11}NO_4$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES• **ORGANIC IMPURITIES**

Protect all solutions from light, and maintain them at 10° until they are injected into the chromatograph.

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Prepare as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area for any impurity from the *Sample solution*

r_S = peak area for levodopa from the *Standard solution*

C_S = concentration of USP Levodopa RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levodopa in the *Sample solution* (mg/mL)

F = relative response factor of the impurity (see *Table 1*)

Acceptance criteria: See Table 1.

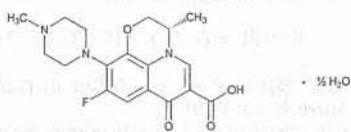
Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levodopa related compound A	0.9	0.83	0.1
Levodopa	1.0	—	—
Levodopa related compound B	2.8	0.83	0.5
5,6-Dihydroxy-indole-2-carboxylic acid	6.0	2.5	0.1
Unknown impurities	—	1.0	0.1 individual 0.3 total unknown
Total impurities	—	—	1.1

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, in a dry place, and prevent exposure to excessive heat.
- **USP REFERENCE STANDARDS** (11)
 - USP Levodopa RS
 - USP Levodopa Related Compound A RS
3-(3,4,6-Trihydroxyphenyl)alanine.
 $C_9H_{11}NO_5$ 213.19
 - USP Levodopa Related Compound B RS
3-Methoxytyrosine.
 $C_{10}H_{13}NO_4$ 211.22

Levofloxacin



$C_{18}H_{20}FN_3O_4 \cdot 1/2H_2O$ 370.38
 7H-Pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid,
 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-
 7-oxo-hydrate (2:1), (S)-;
 (–)-(S)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid, hemihydrate [138199-71-0].
 Anhydrous [100986-85-41].

DEFINITION

Levofloxacin contains NLT 98.0% and NMT 102.0% of $C_{18}H_{20}FN_3O_4$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Buffer: 8.5 g/L of ammonium acetate, 1.25 g/L of cupric sulfate, pentahydrate, and 1.3 g/L of L-isoleucine in water

Mobile phase: Methanol and *Buffer* (3:7)

Standard solution: 1 mg/mL of USP Levofloxacin RS in *Mobile phase*

Sample solution: 1 mg/mL of Levofloxacin in *Mobile phase*

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 360 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 45°

Flow rate: 0.8 mL/min

Injection size: 25 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.5–1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levofloxacin ($C_{18}H_{20}FN_3O_4$) in the portion of Levofloxacin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of levofloxacin from the *Sample solution*

r_s = peak response of levofloxacin from the *Standard solution*

C_s = concentration of USP Levofloxacin RS in the *Standard solution* (mg/mL)

C_u = concentration of Levofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%. Use a platinum crucible.

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 ppm • (Official 1-

Jan-2018)

- **ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—*Procedure 1* is recommended if levofloxacin *N*-oxide is a potential organic impurity. *Procedure 2* and *Procedure 3* are recommended if levofloxacin related compound B is a potential organic impurity.]

Solution A, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

System suitability solution: 1 mg/mL of USP Levofloxacin RS in *Mobile phase*

Sensitivity solution: 0.3 μg/mL of USP Levofloxacin RS in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Relative standard deviation: NMT 1.0%, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual impurity in the portion of Levofloxacin taken:

$$\text{Result} = (r_u/r_s) \times (1/F) \times 100$$

r_u = peak response of each impurity

r_s = peak response of levofloxacin

F = relative response factor (see Table 1)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Desmethyl levofloxacin ^a	0.47	1.0	0.3
Diamine derivative ^b	0.52	0.9	0.3
Levofloxacin N-oxide ^c	0.63	1.1	0.3
9-Desfluoro levofloxacin ^d	0.73	1.0	0.3
Levofloxacin	1.0	—	—
D-Isomer ^e	1.23	1.0	0.8
Any unknown impurity	—	1.0	0.1
Total Impurities	—	—	0.5*

^a (S)-9-Fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

^b (S)-9-Fluoro-2,3-dihydro-3-methyl-10-[2-(methylamino)ethylamino]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

^c (S)-4-(6-Carboxy-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-10-yl)-1-methyl-piperazine-1-oxide.

^d (S)-2,3-Dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

^e (R)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

* Do not include the D-isomer in the calculation for total impurities.

• ORGANIC IMPURITIES, PROCEDURE 2

[NOTE—Solutions of levofloxacin are not stable in light; use amber bottles.]

Buffer: Dissolve 3.08 g/L of ammonium acetate and 8.43 g/L of sodium perchlorate monohydrate in water. Adjust with phosphoric acid to a pH of 2.2.

Solution A: Acetonitrile and *Buffer* (16:84)

Solution B: Acetonitrile, methanol, and *Buffer* (30:20:50)

Solution C: 0.4 mg/mL of USP Levofloxacin RS by dissolving in acetonitrile at about 8% of volume and diluting with water to volume

Solution D: 0.05 mg/mL of USP Levofloxacin Related Compound A RS in 0.2% ammonium hydroxide in methanol

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
10	82	18
15	40	60
30	40	60
30.1	100	0
38	100	0

System suitability solution: 0.1 mg/mL of USP Levofloxacin RS and 5 µg/mL of USP Levofloxacin Related Compound A RS in water from *Solution C* and *Solution D*

Levofloxacin stock solution: 0.4 mg/mL of USP Levofloxacin RS. Dissolve USP Levofloxacin RS in acetonitrile at about 8% of final volume, sonicate, and dilute with water to volume.

Levofloxacin standard solution: 0.02 mg/mL of USP Levofloxacin RS in acetonitrile and water (1:10) from *Levofloxacin stock solution*

Levofloxacin related compound B stock solution: 0.2 mg/mL USP Levofloxacin Related Compound B RS in methanol. [NOTE—Sonicate if necessary.]

Levofloxacin related compound B standard solution: 0.04 mg/mL USP Levofloxacin Related Compound B RS

in methanol from *Levofloxacin related compound B stock solution*

Standard solution: 0.4 µg/mL of levofloxacin and 0.8 µg/mL of levofloxacin related compound B in acetonitrile and water (1:10) from *Levofloxacin standard solution* and *Levofloxacin related compound B standard solution*

Sample solution: 0.4 mg/mL by dissolving the sample in acetonitrile at about 8% of final volume and diluting with water to volume. [NOTE—Sonicate if necessary.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: 280 nm

Column: 4.0-mm × 15-cm; 3.0-µm packing L1

Column temperature: 38°

Flow rate: 1.0 mL/min

Injection size: 10 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Relative standard deviation: NMT 2.0% for levofloxacin

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levofloxacin related compound B in the portion of Levofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for levofloxacin related compound B from the *Sample solution*

r_S = peak response for levofloxacin related compound B from the *Standard solution*

C_S = concentration of USP Levofloxacin Related Compound B RS in the *Standard solution* (mg/mL)

C_U = concentration of Levofloxacin in the *Sample solution* (mg/mL)

Calculate the percentage of other impurities in the portion of Levofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other impurity from the *Sample solution*

r_S = peak response of levofloxacin from the *Standard solution*

C_S = concentration of USP Levofloxacin RS in the *standard solution* (mg/mL)

C_U = concentration of Levofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levofloxacin related compound A (N-Desmethyl levofloxacin) ^a	0.9	0.20
Levofloxacin	1.0	—
Levofloxacin related compound B ^b	2.9	0.13
Any other impurity	—	0.10
Total impurities	—	0.50

^a (S)-9-Fluoro-3-methyl-10-(piperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

^b (S)-9,10-Difluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

• ORGANIC IMPURITIES (ENANTIOMERIC PURITY), PROCEDURE 3

Buffer: 1.32 g/L of D-phenylalanine and 0.75 g/L of copper(II)sulfate pentahydrate in water

Mobile phase: Methanol and Buffer (15:85)

System suitability solution: 0.01 mg/mL of USP Ofloxacin RS and 0.01 mg/mL of USP Levofloxacin RS in water

Sample solution: 0.08 mg/mL in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: 294 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Column temperature: 40°

Flow rate: 0.7 mL/min

Injection size: 10 μL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for D-ofloxacin and levofloxacin are 0.91 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between D-ofloxacin (D-isomer) and levofloxacin

Analysis

Sample: *Sample solution*

Calculate the percentage of D-ofloxacin in the portion of Levofloxacin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for D-ofloxacin

r_T = sum of responses of all peaks

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

• **OPTICAL ROTATION, Specific Rotation (781S)**

Solvent: Methanol

Sample solution: 5 mg/mL in Solvent

Acceptance criteria: −92° to −106°, at 20°

• **WATER DETERMINATION, Method 1a (921):** 2.0%–3.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.

• **LABELING:** If a procedure for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* procedure the article complies.

• **USP REFERENCE STANDARDS (11)**

USP Levofloxacin RS

USP Levofloxacin Related Compound A RS

(S)-9-Fluoro-3-methyl-10-(piperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

$C_{17}H_{18}FN_3O_4$ 347.34

USP Levofloxacin Related Compound B RS

(S)-9,10-Difluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

$C_{13}H_9F_2NO_4$ 281.21

USP Ofloxacin RS

Levofloxacin Oral Solution

DEFINITION

Levofloxacin Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

[NOTE—Protect the solutions of levofloxacin from light.]

Diluent: Acetonitrile and water (18:82)

Mobile phase: *Diluent* that contains 1 mL of trifluoroacetic acid in each 1000 mL of solution

Standard solution: 102.5 μg/mL of USP Levofloxacin RS in *Diluent*

System suitability solution: 102.5 μg/mL each of USP Levofloxacin RS and USP Levofloxacin Related Compound A RS in *Diluent*

Sample solution: 102.5 μg/mL of levofloxacin in *Diluent* based on the label claim. [NOTE—Mix the solution well after equilibrating the solution for 4 h at room temperature while protected from light.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 294 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Column temperature: 30°

Flow rate: 0.7 mL/min

Run time: 2.5 times the retention time of the levofloxacin peak

Injection size: 20 μL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 1.9 between levofloxacin related compound A and levofloxacin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Levofloxacin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Organic Impurities

• **PROCEDURE**

[NOTE—Protect the solutions of levofloxacin from light.]

Diluent, Mobile phase, Standard solution, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of levofloxacin from the *Standard solution*

C_S = concentration of USP Levofloxacin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of levofloxacin in the *Sample solution* (mg/mL)

F = relative response factor for each impurity (See *Impurity Table 1*)

Acceptance criteria

Individual impurities: See Impurity Table 1.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
9-Desfluoro levofloxacin ^a	0.64	1.0	— [*]
Diamine derivative ^b	0.75	1.0	— [*]
Levofloxacin related compound A ^c	0.91	0.81	0.5
Levofloxacin	1.0	—	—
Levofloxacin N-oxide ^d	1.55	0.93	0.5
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	1.0

^a (S)-2,3-Dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.^b (S)-9-Fluoro-2,3-dihydro-3-methyl-10-[2-(methylamino)ethylamino]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.^c (S)-9-Fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.^d (S)-4-(6-Carboxy-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-10-yl)-1-methylpiperazine 1-oxide.^{*} Disregard this peak because this is a process impurity controlled for the drug substance.

SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed 10^2 cfu/mL, and the total combined molds and yeast count does not exceed 10^1 cfu/mL. It also meets the requirement for absence of *Escherichia coli*.
- DELIVERABLE VOLUME (698):** Meets the requirements
- PH (791):** 5.0–6.0

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Store at controlled room temperature, and protect from light.
- USP REFERENCE STANDARDS (11)**
 - USP Levofloxacin RS
 - 7H-Pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-hydrate (2:1), (S)-;
 - (-)- (S)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid, hemihydrate.
 - Anhydrous.
 - $C_{18}H_{20}FN_3O_4 \cdot \frac{1}{2}H_2O$ 370.38
 - USP Levofloxacin Related Compound A RS
 - (S)-9-Fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.
 - $C_{17}H_{18}FN_3O_4$ 347.34

Levofloxacin Tablets

DEFINITION

Levofloxacin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$).

IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Diluent: Acetonitrile and water (20:80)**Mobile phase:** Transfer 874 mg of cupric sulfate, 918 mg of L-isoleucine, and 5.94 g of ammonium acetate to a suitable container. Add 700 mL of water, and mix until dissolved. Add 300 mL of methanol.**Standard stock solution:** 2 mg/mL of USP Levofloxacin RS in *Diluent***Standard solution:** 0.2 mg/mL of USP Levofloxacin RS in *Mobile phase* from the *Standard stock solution***Sample stock solution:** Nominally 5 mg/mL of levofloxacin prepared as follows. Transfer intact Tablets (NLT 5) to a volumetric flask, add 75% of the final volume of *Diluent*, and allow to stand for 15 min. Shake for 30 min, and dilute with *Diluent* to volume. Pass a portion of the solution through a suitable filter of 0.45- μ m pore size, discarding the first 1–2 mL of the filtrate.**Sample solution:** Nominally 0.2 mg/mL of levofloxacin in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC**Detector:** UV 360 nm**Column:** 4.6-mm \times 25-cm; 5- μ m packing L1**Column temperature:** 45°**Flow rate:** 0.8 mL/min**Injection volume:** 25 μ L**Run time:** 2 times the retention time of levofloxacin

System suitability

Sample: *Standard solution***Suitability requirements****Tailing factor:** NMT 1.8**Relative standard deviation:** NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of levofloxacin from the *Sample solution* r_S = peak response of levofloxacin from the *Standard solution* C_S = concentration of USP Levofloxacin RS in the *Standard solution* (mg/mL) C_U = nominal concentration of levofloxacin in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL**Apparatus 2:** 75 rpm**Time:** 30 min**Standard solution:** 0.56 mg/mL of USP Levofloxacin RS in *Medium***Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

Mode: UV**Analytical wavelength:** 294 nm**Cell length:** 0.1 mm**Blank:** *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*Calculate the percentage (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)
 V = volume of *Medium*, 900 mL
 D = dilution factor of the *Sample solution*
 L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) is dissolved.

Test 2

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: L/900 mg/mL of USP Levofloxacin RS in *Medium*, and mix to obtain solutions with known concentrations as indicated in *Table 1*, where L is the label claim in mg/Tablet.

Sample solution: Pass a portion of the solution under test having a concentration similar to that of the *Standard solution* through a suitable filter of 0.45- μ m pore size.

Table 1

Tablet Label Claim (mg)	Final Concentration (mg/mL)
250	0.27
500	0.55
750	0.83

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 293 nm

Cell length: 0.1 mm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)
 V = volume of *Medium*, 900 mL
 D = dilution factor of the *Sample solution*
 L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) is dissolved.

Test 3

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: L/900 mg/mL of USP Levofloxacin RS in *Medium*, and mix to obtain solutions with known concentrations as indicated in *Table 1*, where L is the label claim in mg/Tablet.

Sample solution: Pass a portion of the solution under test having the same concentration as that of the *Standard solution* through a suitable filter of 0.45- μ m pore size.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 326 nm

Cell length: 1 mm for a 250-mg Tablet, 0.5 mm for a 500-mg Tablet, and 0.2 mm for a 750-mg Tablet

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)
 V = volume of *Medium*, 900 mL
 D = dilution factor of the *Sample solution*
 L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) is dissolved.

Test 4

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: 16 μ g/mL of USP Levofloxacin RS in *Medium*

Sample solution: Pass a portion of the solution under test having the same concentration as that of the *Standard solution* through a suitable filter of 0.45- μ m pore size.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 332 nm

Cell length: 1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)
 V = volume of *Medium*, 900 mL
 D = dilution factor of the *Sample solution*
 L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

IMPURITIES• **ORGANIC IMPURITIES**

Diluent, Mobile phase, Standard stock solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.2 mg/mL of USP Levofloxacin RS from the *Standard stock solution* and 1 μ g/mL of USP Levofloxacin Related Compound A RS in *Mobile phase*

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.8 for the levofloxacin peak

Relative standard deviation: NMT 2.0% for the levofloxacin peak

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of levofloxacin related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of levofloxacin related compound A from the *Sample solution*

- r_s = peak response of levofloxacin related compound A from the *Standard solution*
 C_s = concentration of USP Levofloxacin Related Compound A RS in the *Standard solution* (mg/mL)
 C_u = nominal concentration of levofloxacin in the *Sample solution* (mg/mL)
 Calculate the percentage of any other impurities in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

- r_u = peak response of any impurity from the *Sample solution*
 r_s = peak response of levofloxacin from the *Standard solution*
 C_s = concentration of USP Levofloxacin RS in the *Standard solution* (mg/mL)
 C_u = nominal concentration of levofloxacin in the *Sample solution* (mg/mL)
 F = relative response factor (see Table 2)
 Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Decarboxy levofloxacin ^a	0.38	0.60	0.3
Levofloxacin related compound A ^b	0.47	—	0.7
Diamine derivative ^c	0.52	0.83	0.3
Levofloxacin N-oxide ^d	0.63	0.68	0.7
9-Desfluoro levofloxacin ^e	0.73	—	—
Levofloxacin	1.00	—	—
Dextroflaxacin ^g	1.23	—	—
Levofloxacin 9-piperazino isomer ^h	1.69	—	—
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	1

^a (S)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine.

^b (S)-9-Fluoro-3-methyl-10-(piperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

^c (S)-9-Fluoro-2,3-dihydro-3-methyl-10-[2-(methylamino)ethylamino]-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

^d (S)-4-(6-Carboxy-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-10-yl)-1-methylpiperazine 1-oxide.

^e (S)-2,3-Dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

^f Process impurity, for information only.

^g (R)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

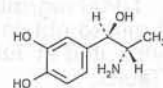
^h (S)-10-fluoro-3-methyl-9-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

- USP REFERENCE STANDARDS (11)**
 USP Levofloxacin RS
 USP Levofloxacin Related Compound A RS
 (S)-9-Fluoro-3-methyl-10-(piperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.
 $C_{17}H_{18}FN_3O_4$ 347.34

Levonordefrin



$C_9H_{13}NO_3$ 183.20
 1,2-Benzenediol, 4-(2-amino-1-hydroxypropyl)-, [*R*-(*R**,*S**)]-(-)- α -(1-Aminoethyl)-3,4-dihydroxybenzyl alcohol
 [18829-78-2; 829-74-3].

» Levonordefrin, dried in vacuum at 60° for 15 hours, contains not less than 98.0 percent and not more than 102.0 percent of $C_9H_{13}NO_3$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Levonordefrin RS

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 25 μ g per mL.

Medium: 0.1 N hydrochloric acid.

Specific rotation (781S): between -28° and -31°.

Test solution: 50 mg, previously dried, per mL, in 0.3 N hydrochloric acid.

Loss on drying (731)—Dry it in vacuum at 60° for 15 hours: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.2%.

Chromatographic purity—

Standard solutions—Dissolve an accurately weighed quantity of USP Levonordefrin RS in a mixture of methanol and glacial acetic acid (96:4) to obtain a Standard stock solution having a known concentration of 5 mg per mL. Dilute this solution quantitatively with a mixture of methanol and glacial acetic acid (96:4) to obtain *Standard solutions*, designated below by letter, having the following compositions:

Standard solution	Dilution	Concentration (μ g RS per mL)	Percentage (% for comparison with test specimen)
A	(1 in 10)	500	1.0
B	(1 in 20)	250	0.5
C	(1 in 50)	100	0.2
D	(1 in 100)	50	0.1

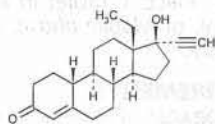
Test solution—Dissolve an accurately weighed quantity of Levonordefrin in a mixture of methanol and glacial acetic acid (96:4) to obtain a solution containing 50 mg per mL.

Procedure—Apply separately 5 μ L of the *Test solution* and 5 μ L of each *Standard solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of *n*-butyl alcohol, water, and glacial acetic acid.

(70:20:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in warm, circulating air. Examine the plate under short-wavelength UV light. Expose the plate to iodine vapors, and examine again. Compare the intensities, observed by both visualizations, of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions*: the sum of the intensities of secondary spots obtained from the *Test solution* corresponds to not more than 1.0% of related compounds, with no single impurity corresponding to more than 0.5%.

Assay—Transfer about 350 mg of Levonorgestrel, previously dried and accurately weighed, to a small flask, dissolve in 50 mL of glacial acetic acid, heating, if necessary, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 18.32 mg of $C_{21}H_{28}O_2$.

Levonorgestrel



$C_{21}H_{28}O_2$ 312.45

18,19-Dinorpregn-4-en-20-yn-3-one, 13-ethyl-17-hydroxy-, (17 α)-(-)-

(-)-13-Ethyl-17-hydroxy-18,19-dinor-17 α -pregn-4-en-20-yn-3-one [797-63-7].

» Levonorgestrel contains not less than 98.0 percent and not more than 102.0 percent of $C_{21}H_{28}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards (11)—

USP Levonorgestrel RS

Identification—

A: *Infrared Absorption* (197K).

B: Meeting the requirements of the tests for *Specific rotation* and *Melting range* provides identification distinguishing it from norgestrel.

Melting range (741): between 232° and 239°, but the range between beginning and end of melting does not exceed 4°.

Specific rotation (781S): between -30° and -35°.

Test solution: 20 mg per mL, in chloroform.

Loss on drying (731)—Dry it at 105° for 5 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.3%.

Limit of ethynyl group—Dissolve 200 mg in about 40 mL of tetrahydrofuran. Add 10 mL of silver nitrate solution (1 in 10), and titrate with 0.1 N sodium hydroxide VS, using either a glass-calomel or a silver-silver chloride electrode system with potassium nitrate filling solution. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 2.503 mg of ethynyl group ($-C\equiv CH$). Not less than 7.81% and not more than 8.18% of ethynyl group is found.

Chromatographic purity—Proceed as directed in the test for *Chromatographic purity* under *Norgestrel*, using USP Levo-

norgestrel RS in place of USP Norgestrel RS. The requirements of the test are met if the sum of the impurities in the *Test preparation* does not exceed 2.0% and no single impurity is greater than 0.5%.

Assay—Using USP Levonorgestrel RS, proceed as directed in the *Assay* under *Norgestrel*, except to read "Levonorgestrel" in place of "Norgestrel."

Levonorgestrel and Ethinyl Estradiol Tablets

DEFINITION

Levonorgestrel and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of levonorgestrel ($C_{21}H_{28}O_2$) and NLT 90.0% and NMT 110.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

- A. The retention times of the two major peaks of the *Sample solution* correspond to those of levonorgestrel and ethinyl estradiol in the *Standard solution*, as obtained in the *Assay*.
 - B. Finely powder 20 Tablets and transfer a portion of the powder, equivalent to 4 mg of levonorgestrel, to a suitable container. Add 250 mL of a solvent mixture consisting of isooctane and chloroform (3:1). Sonicate the mixture for 3 min, and then stir it by mechanical means for 30 min. Filter the mixture and evaporate the filtrate to dryness in a rotating vacuum evaporator. Dissolve the residue in 3 mL of chloroform, and transfer with a pipet to a 60-mL separator containing 18 mL of isooctane. Rinse the evaporator flask with an additional 3-mL portion of chloroform, and add the rinsing to the separator. Add 10 mL of 1 N sodium hydroxide, shake vigorously, and allow the layers to separate. Discard the lower aqueous phase, and filter the organic phase through 3 g of anhydrous sodium sulfate on filter paper into a 50-mL beaker. Rinse the filter with several small portions of the mixture of isooctane and chloroform (3:1), adding the filtered rinsings to the filtrate, and evaporate under nitrogen on a steam bath to dryness. Dissolve the residue in 1–2 mL of hot toluene, and transfer with a pipet to a small glass vial. Reduce the volume of the solution to 0.1 mL under nitrogen with warming. [NOTE—During this step, any crystals that deposit on the vial wall should be transferred to the bottom, and allowed to redissolve.] Store the vial containing the clear toluene solution at 4° overnight to allow crystallization to occur. Remove and discard the mother liquor with a pipet, rinse the crystals with two 0.5-mL portions of anhydrous ether, and discard the rinsings. Dry the vial containing the rinsed crystals in a vacuum desiccator at 60° for 4 h.
- Acceptance criteria:** The melting point of the dried crystals of levonorgestrel so obtained is not lower than 220°, using the procedure described under *Melting Range or Temperature* (741), *Class I*.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile, methanol, and water (35:15:45)

Standard solution: 15 μ g/mL of USP Levonorgestrel RS and 3 μ g/mL of USP Ethinyl Estradiol RS in *Mobile phase*

Sample solution: Transfer a number of Tablets, equivalent to 3 mg of levonorgestrel, to a 200-mL volumetric flask. Dilute with *Mobile phase* to volume, sonicate to disintegrate the Tablets, then shake by mechanical means for 20 min. Centrifuge, and use the clear supernatant.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5- to 7-μm packing L7

Flow rate: 1 mL/min

Injection size: 50 μL

System suitabilitySample: *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol and levonorgestrel are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between the two major peaks

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of C₂₁H₂₈O₂ and C₂₀H₂₄O₂ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*r_S = peak response of the corresponding analyte from the *Standard solution*C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (μg/mL)C_U = nominal concentration of the corresponding analyte in the *Sample solution* (μg/mL)Acceptance criteria: 90.0%–110.0% of the labeled amount of C₂₁H₂₈O₂, 90.0%–110.0% of the labeled amount of C₂₀H₂₄O₂**PERFORMANCE TESTS**

- **DISSOLUTION (711):** Determine the amount of C₂₁H₂₈O₂ and C₂₀H₂₄O₂ dissolved by employing the following method.

Medium: Polysorbate 80 (5 μg/g) in water; 500 mL

Apparatus 2: 75 rpm

Time: 60 min

Mobile phase: Acetonitrile and water (6:4)

Standard solution: Prepare a solution of USP Levonorgestrel RS and USP Ethinyl Estradiol RS in *Medium* having known concentrations corresponding approximately to the concentrations that would be obtained by dissolving 1 Tablet in 500 mL of *Medium*.

[NOTE—A volume of alcohol not exceeding 2% of the final total volume of solution may be used to aid in dissolving the Reference Standards.]

Sample solution: Withdraw 15-mL portions of liquid from each vessel, and pass through a polyvinylidene filter, discarding the first 10 mL of the filtrate.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 247 nm (for levonorgestrel analysis); a spectrofluorometric detector (for ethinyl estradiol analysis), with an excitation wavelength of 285 nm, and an emission wavelength of 310 nm

Column: 4-mm × 15-cm; packing L7

Flow rate: 1 mL/min

Injection size: 100 μL

System suitabilitySample: *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol and levonorgestrel are about 0.7 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 3.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of C₂₁H₂₈O₂ and C₂₀H₂₄O₂ dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*r_S = peak response of the corresponding analyte from the *Standard solution*C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (μg/mL)C_U = nominal concentration of the corresponding analyte in the *Sample solution* (μg/mL)**Tolerances**Uncoated Tablets: NLT 80% (Q) of the labeled amount of C₂₁H₂₈O₂, and 75% (Q) of the labeled amount of C₂₀H₂₄O₂ is dissolved.Coated Tablets: NLT 60% (Q) of the labeled amount of C₂₁H₂₈O₂ and C₂₀H₂₄O₂ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

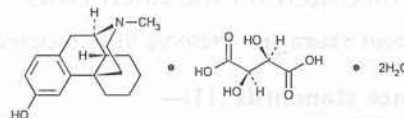
Sample solution: Place 1 Tablet in a 40-mL centrifuge tube, add 10.0 mL of *Mobile phase*, and proceed as directed in the *Assay*.**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**

USP Ethinyl Estradiol RS

USP Levonorgestrel RS

Levorphanol TartrateC₁₇H₂₃NO · C₄H₆O₆ · 2H₂O 443.49

Morphinan-3-ol, 17-methyl-, [R-(R*,R*)]-2,3-dihydroxybutanedioate (1:1) (salt), dihydrate.

17-Methylmorphinan-3-ol, tartrate (1:1) (salt) dihydrate [5985-38-6].

Anhydrous 407.47 [125-72-4].

» Levorphanol Tartrate contains not less than 99.0 percent and not more than 101.0 percent of C₁₇H₂₃NO · C₄H₆O₆, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards (11)

USP Levorphanol Tartrate RS

Identification

A: Infrared Absorption (197K)—Obtain the test specimen as follows. Dissolve 50 mg in 25 mL of water in a 125-mL separator. Add 2 mL of 6 N ammonium hydroxide, extract with 25 mL of chloroform, and filter the chloroform extract through a layer of 4 g of granular anhydrous sodium sulfate supported on glass wool into a 125-mL conical flask. Evaporate the chloroform extract on a steam bath with the aid of

a stream of nitrogen to dryness. Dissolve the residue in 1 mL of acetone, and evaporate to dryness. Dry in vacuum at 90° for 1 hour. Proceed as directed with the dried levorphanol so obtained and a similar preparation of USP Levorphanol Tartrate RS.

B: Ultraviolet Absorption (197U)—

Solution: 130 µg per mL.

Medium: 0.1 N hydrochloric acid.

Absorptivities at 279 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

Specific rotation (781S): between -14.7° and -16.3°.

Test solution: 30 mg per mL, in water. Heat on a water bath or sonicate to dissolve 750 mg in 20 mL of water in a 25-mL volumetric flask, dilute with water to volume, and mix.

Water Determination, Method I (921): between 7.0% and 9.0%.

Residue on ignition (281): not more than 0.1%.

Ordinary impurities (466)—

Test solution: water.

Standard solution: water.

Eluent: a mixture of hexanes, dehydrated alcohol, and ammonium hydroxide (80:25:1).

Visualization: 17; then view immediately under short-wavelength UV light.

Assay—Dissolve about 900 mg of sample, accurately weighed, in 85 mL of glacial acetic acid, warming slightly if necessary. Titrate with 0.1 N perchloric acid VS and determine the endpoint potentiometrically. Perform a blank determination and make any necessary corrections. Each mL of 0.1 N perchloric acid consumed by the sample is equivalent to 40.75 mg of $C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$.

Levorphanol Tartrate Injection

» Levorphanol Tartrate Injection is a sterile solution of Levorphanol Tartrate in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards (11)—

USP Endotoxin RS

Identification—

A: To 1 mL of Injection add 1 drop of 3 N hydrochloric acid and 2 drops of ferric chloride TS. Heat to boiling, and add 1 mL of potassium ferricyanide solution (1 in 200): a blue-green color develops.

B: The angular rotation of the Injection is levorotatory (see *Optical Rotation* (781)).

Bacterial Endotoxins Test (85)—It contains not more than 125.0 USP Endotoxin Units per mg of levorphanol tartrate.

pH (791): between 4.1 and 4.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—Transfer an accurately measured volume of Injection, equivalent to about 40 mg of levorphanol tartrate, to a 125-mL separator. Add 5 g of sodium chloride and sufficient sodium bicarbonate to render the solution alkaline to litmus, add an additional 100 mg of sodium bicarbonate, and extract the levorphanol with five 20-mL portions of a mixture

of 3 volumes of ether and 1 volume of chloroform. Pass the combined extracts through a layer of about 10 g of granular anhydrous sodium sulfate into a 500-mL conical flask, and evaporate to a volume of about 30 mL. Add about 50 mL of chloroform and 1 drop of methanolic methyl red TS, and titrate with 0.01 N perchloric acid in dioxane VS to a red endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.01 N perchloric acid is equivalent to 4.435 mg of $C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$.

Levorphanol Tartrate Tablets

» Levorphanol Tartrate Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of levorphanol tartrate ($C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Levorphanol Tartrate RS

Identification—

A: Powder finely a number of Tablets. To a portion of the powder, equivalent to about 1 mg of levorphanol tartrate, add 1 mL of water, 1 drop of 3 N hydrochloric acid, and 2 drops of ferric chloride TS, and heat to boiling. To the hot solution add 1 mL of potassium ferricyanide solution (1 in 200): a bluish color develops.

B: Powder a number of Tablets, equivalent to about 60 mg of levorphanol tartrate, and transfer the mixture to a small separator. Add 10 mL of water, dissolve as much of the powder as possible, add about 400 mg of sodium bicarbonate, and extract with a 50-mL portion of chloroform. Evaporate the filtered chloroform extract on a steam bath to a small volume, dilute with chloroform to 10 mL, and determine the angular rotation: the solution is levorotatory (see *Optical Rotation* (781)).

Dissolution (711)—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 279 nm on filtered portions of the solution under test, suitably diluted with water, in comparison with a Standard solution having a known concentration of USP Levorphanol Tartrate RS in the same medium.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Procedure for content uniformity—Transfer 1 Tablet to a glass-stoppered flask, add 25.0 mL of 0.1 N hydrochloric acid, and allow the Tablet to disintegrate. Shake well, and filter through a small filter paper, discarding the first portion of the filtrate. Dilute a portion of the filtrate quantitatively and stepwise, if necessary, to provide a solution containing about 80 µg of levorphanol tartrate per mL. Concomitantly determine the absorbances of this solution and of a solution of USP Levorphanol Tartrate RS in the same medium having a known concentration of about 80 µg of anhydrous levorphanol tartrate per mL, in 1-cm cells at the wavelength of maximum absorbance at about 279 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the

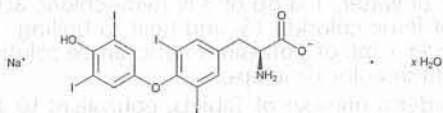
blank. Calculate the quantity, in mg, of $C_{17}H_{23}NO \cdot C_4H_6O_6 \cdot 2H_2O$ in the Tablet taken by the formula:

$$(443.49 / 407.47)(TC / D)(A_U / A_S)$$

in which 443.49 and 407.47 are the molecular weights of the hydrated and anhydrous forms of levorphanol tartrate, respectively; T is the labeled quantity, in mg, of levorphanol tartrate in the Tablet; C is the concentration, in μg per mL, of USP Levorphanol Tartrate RS, on the anhydrous basis, in the Standard solution; D is the concentration, in μg per mL, of levorphanol tartrate in the solution from the Tablet, based on the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 40 mg of levorphanol tartrate, transfer to a 125-mL separator, add 20 mL of water and sufficient sodium bicarbonate to render the suspension alkaline to litmus, and proceed as directed in the Assay under *Levorphanol Tartrate Injection*, beginning with "add an additional 100 mg of sodium bicarbonate."

Levothyroxine Sodium



$C_{15}H_{10}I_4NNaO_4 \cdot xH_2O$ (anhydrous) 798.85
L-Tyrosine, O-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-, monosodium salt, hydrate;
Monosodium L-thyroxine hydrate [25416-65-3].
Anhydrous [55-03-8].

DEFINITION

Levothyroxine Sodium is the sodium salt of L-3,3',5,5'-tetraiodothyronine. It contains NLT 97.0% and NMT 103.0% of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197): [NOTE—Methods described in *Infrared Absorption* (197K) or (197A) may be used.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **C. IDENTIFICATION TESTS—GENERAL, Sodium** (191)
Sample solution: To 200 mg add 2 mL of 2 N sulfuric acid. Heat on a water bath and then carefully heat over an open flame, increasing the temperature gradually up to about 600°. [NOTE—Alternative procedures for igniting the material could also be used.] Continue the ignition until most of the particles have disappeared. Dissolve the residue in 2 mL of water.
Acceptance criteria: The *Sample solution* meets the requirements of the pyroantimonate precipitation test.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile and water (4:6) that contains 0.5 mL of phosphoric acid in each 1000 mL

Solution A: 400 mg of sodium hydroxide in 500 mL of water. Cool and add 500 mL of methanol.

Levothyroxine stock solution: 0.4 mg/mL of USP Levothyroxine RS in *Solution A*

Liothyronine stock solution: 0.4 mg/mL of liothyronine from USP Liothyronine RS in *Solution A*. Make a 1:100 dilution of this solution using *Mobile phase*.

Standard solution: 10 μg /mL of levothyroxine from *Levothyroxine stock solution* and 0.2 μg /mL of liothyronine from *Liothyronine stock solution* in *Mobile phase*

Sample solution: 10 μg /mL of Levothyroxine Sodium in *Mobile phase*. [NOTE—A small amount of 0.01 M methanolic sodium hydroxide can be used to facilitate the dissolution of the sample.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm \times 25-cm; packing L10

Flow rate: 1.5 mL/min

Injection volume: 100 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between liothyronine and levothyroxine

Relative standard deviation: NMT 2.0% for levothyroxine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of levothyroxine from the *Sample solution*

r_S = peak response of levothyroxine from the *Standard solution*

C_S = concentration of USP Levothyroxine RS in the *Standard solution* ($\mu\text{g}/\text{mL}$)

C_U = concentration of Levothyroxine Sodium in the *Sample solution* ($\mu\text{g}/\text{mL}$)

M_{r1} = molecular weight of levothyroxine sodium, 798.85

M_{r2} = molecular weight of levothyroxine, 776.87

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

[NOTE—On the basis of the synthetic route, perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*. *Procedure 2* is recommended when related compounds listed in *Table 3* may be present.]

• ORGANIC IMPURITIES, PROCEDURE 1

Diluent: Acetonitrile and water (1:1)

Solution A: Dilute 5 mL of phosphoric acid with *Diluent* to 100.0 mL.

Mobile phase: Dissolve 1.0 g of sodium 1-heptanesulfonate in 200 mL of water. Add 200 mL of acetonitrile, 400 mL of methanol, and 1.0 mL of phosphoric acid. Dilute with water to 1 L.

Standard stock solution 1: Transfer 25 mg of USP Levothyroxine RS to a 100-mL volumetric flask. Add 50 mL of *Diluent* and 1 drop of 10 N sodium hydroxide, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Standard stock solution 2: Transfer 25 mg of USP Liothyronine RS to a 100-mL volumetric flask. Add 50 mL of *Diluent* and 1 drop of 10 N sodium hydroxide, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

System suitability solution: Transfer 5.0 mL of *Standard stock solution 1* and 5.0 mL of *Standard stock solution 2* to a 100-mL volumetric flask. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Standard solution: Pipet 4.0 mL of the *System suitability solution* into a 100-mL volumetric flask. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Sample solution: Transfer 25 mg of Levothyroxine Sodium to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and sonicate until dissolved. Add 7 mL of *Solution A*, and dilute with *Diluent* to volume.

Blank solution: Transfer 7 mL of *Solution A* to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 15 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between levothyroxine and liothyronine, *System suitability solution*

Relative standard deviation: NMT 2.0% for the levothyroxine peak, *Standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank solution*

[NOTE—Record the chromatograms for at least six times the retention time of the levothyroxine peak. Verify that no peaks elute in the *Blank solution* at the expected retention times for levothyroxine and related compounds.]

Calculate the area percentage of each related compound in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of levothyroxine from the *Standard solution*

C_S = concentration of USP Levothyroxine RS in the *Standard solution* (mg/mL)

C_U = concentration of Levothyroxine Sodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of levothyroxine sodium, 798.85

M_{r2} = molecular weight of levothyroxine, 776.87

[NOTE—The relative response factor for the impurities listed in *Table 1* is 1.00. Any unspecified impurity peaks should be assigned a relative response factor of 1.00.]

Disregard peaks corresponding to those of the *Blank solution*, and disregard peaks corresponding to less than 0.03%.

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Liothyronine	0.65–0.70	1.0
β-Hydroxy-T4 ^a	0.71–0.76	0.15

^a O-(4-Hydroxy-3,5-diiodophenyl)-3,5-diiodo-β-hydroxy-L-tyrosine.

^b 2-Hydroxy-2-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetic acid.

^c N-Formyl-O-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.

^d 2-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetamide.

^e N-Acetyl-O-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.

^f 2-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetic acid.

^g 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzaldehyde.

^h 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzoic acid.

Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levothyroxine	1.0	—
T4-Hydroxyacetic acid ^b	1.13–1.28	0.15
N-Formyl-T4 ^c and T4-acetamide ^d	1.47–1.53	0.15
N-Acetyl-T4 ^e	1.50–1.86	0.20
T4-Acetic acid ^f	2.42–2.51	0.30
T4-Aldehyde ^g	3.17–3.45	0.15
T4-Benzoic acid ^h	3.46–3.70	0.15
Individual unspecified impurity	—	0.10
Total impurities	—	2.0

^a O-(4-Hydroxy-3,5-diiodophenyl)-3,5-diiodo-β-hydroxy-L-tyrosine.

^b 2-Hydroxy-2-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetic acid.

^c N-Formyl-O-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.

^d 2-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetamide.

^e N-Acetyl-O-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.

^f 2-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetic acid.

^g 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzaldehyde.

^h 4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzoic acid.

• ORGANIC IMPURITIES, PROCEDURE 2

Solution A: Dissolve 9.7 g of sulfamic acid in 2000 mL of water. Add 1.5 g of sodium hydroxide, mix to dissolve, and adjust with 2 N sodium hydroxide to a pH of 2.0.

Solution B: Acetonitrile

Diluent 1: Methanol and *Solution A* (90:10)

Diluent 2: Acetonitrile and *Solution A* (30:70); mix with *Diluent 1* (1:1).

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	70	30
10	70	30
40	20	80
50	20	80
53	70	30
75	70	30

Identification solution: Dissolve 5.0 mg of USP Levothyroxine for Peak Identification RS in 4.5 mL of methanol. Add 0.5 mL of *Solution A*. Further dilute a portion of this solution with *Diluent 2* to obtain a solution containing about 0.2 mg/mL.

Standard stock solution: 0.1 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS in *Diluent 1*

Standard solution: 0.002 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS, prepared using the *Standard stock solution* in *Diluent 2*

Sensitivity solution: 0.0002 mg/mL each of USP Levothyroxine RS and USP Liothyronine RS, prepared using the *Standard solution* in *Diluent 2*

Sample solution: Dissolve an amount of Levothyroxine Sodium in *Diluent 1* to obtain a solution with a known concentration of about 1.0 mg/mL. Further dilute a portion of this solution with *Diluent 2* to obtain a solution with a known concentration of about 0.2 mg/mL.

Blank solution: Use Diluent 2.

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV 225 nm

Column: 4.0-mm × 15-cm; 3-μm packing L1

Flow rate: 1.0 mL/min

Injection volume: 25 μL

System suitability

Samples: Standard solution and Sensitivity solution

Suitability requirements

Resolution: NLT 5 between levothyroxine and liothyronine, Standard solution

Signal-to-noise ratio: NLT 5 for each peak from the Sensitivity solution, calculated as follows:

$$\text{Result} = (2H)/h$$

H = measured height of the peak

h = amplitude of the average measured baseline noise

Analysis

Samples: Blank solution, Standard solution, Identification solution, and Sample solution

[NOTE—Identify the components on the basis of their relative retention times as listed in Table 3.]

Calculate the percentage of liothyronine sodium in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of liothyronine from the Sample solution

r_S = peak response of liothyronine from the Standard solution

C_S = concentration of USP Liothyronine RS in the Standard solution (mg/mL)

C_U = concentration of Levothyroxine Sodium in the Sample solution (mg/mL)

M_{r1} = molecular weight of liothyronine sodium, 672.96

M_{r2} = molecular weight of liothyronine, 650.98
Calculate the percentage of any other impurity in the portion of Levothyroxine Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of any impurity from the Sample solution

r_S = peak response of levothyroxine from the Standard solution

C_S = concentration of USP Levothyroxine RS in the Standard solution (mg/mL)

C_U = concentration of Levothyroxine Sodium in the Sample solution (mg/mL)

M_{r1} = molecular weight of levothyroxine sodium, 798.85

M_{r2} = molecular weight of levothyroxine, 776.87

[NOTE—The relative response factor for the impurities listed in Table 3 is 1.00. Any unspecified impurity peaks should be assigned a relative response factor of 1.00.]

Disregard peaks corresponding to those of the Blank solution, and disregard peaks corresponding to less than 0.03%.

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Liothyronine	0.65	1.0
Monochlorotriiodothyronine ^a	0.94	0.15
Levothyroxine N-methylamide ^b	0.97	0.15
Levothyroxine	1.0	—
Triiodothyroacetic acid, or T3-acetic acid ^c	1.57	0.15
O-(4-Hydroxy-3,5-diiodophenyl)thyroxine, or T6 ^d	1.61	0.50
O-Methyl-tetraiodothyroethylamine, or T4-amine O-methyl ^e	1.76	0.30
T4-Acetic acid ^f	1.79	0.30
Individual unspecified impurity	—	0.10
Total impurities	—	2.0

^a (S)-2-Amino-3-[3-chloro-4-(4-hydroxy-3,5-diiodophenoxy)-5-iodophenyl]-propanoic acid.

^b (S)-2-Amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]-N-methylpropanamide.

^c [4-(4-Hydroxy-3-iodophenoxy)-3,5-diiodophenyl]acetic acid.

^d (S)-2-Amino-3-[4-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenoxy]-3,5-diiodophenyl]propanoic acid.

^e 2-[4-(3,5-Diiodo-4-methoxyphenoxy)-3,5-diiodophenyl]ethanamine.

^f 2-(4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl)acetic acid.

SPECIFIC TESTS

• OPTICAL ROTATION, Specific Rotation (781S)

Sample solution: Equivalent to 30 mg/mL of anhydrous Levothyroxine Sodium in alcohol and 1 N sodium hydroxide (2:1)

Acceptance criteria: -5° to -6°

• WATER DETERMINATION, Method I (921): NMT 11.0%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in tight containers, protected from light. Store as stated in the labeling instructions.

• LABELING: If a test for Organic Impurities other than Procedure 1 is used, the labeling states the test with which the article complies.

• USP REFERENCE STANDARDS (11)

USP Levothyroxine RS

O-(4-Hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine.

$C_{15}H_{11}I_4NO_4$ 776.87

USP Levothyroxine for Peak Identification RS

Levothyroxine sodium spiked with liothyronine, triiodothyroacetic acid, and tetraiodothyroacetic acid.

USP Levothyroxine Sodium RS

USP Liothyronine RS

O-(4-Hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosine.

$C_{15}H_{12}I_3NO_4$ 650.98

Levothyroxine Sodium Oral Powder

» Levothyroxine Sodium Oral Powder contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$).

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Levothyroxine RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Loss on drying (731)—Dry it in vacuum at 60° for 3 hours; it loses not more than 2.0% of its weight.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (65:35) that contains 1 mL of phosphoric acid in each 1000 mL. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

0.01 M Methanolic sodium hydroxide—Dissolve 400 mg of sodium hydroxide in 500 mL of water. Cool, add 500 mL of methanol, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Levothyroxine RS in 0.01 M Methanolic sodium hydroxide, and dilute quantitatively and stepwise with 0.01 M Methanolic sodium hydroxide to obtain a solution having a known concentration of about 4 µg per mL.

Assay preparation—Transfer an accurately weighed portion of Oral Powder, equivalent to about 5 mg of levothyroxine sodium, to a 250-mL volumetric flask. Dilute with 0.01 M Methanolic sodium hydroxide to volume, mix, and allow to stand for 4 hours, with occasional mixing. Pass a portion of this mixture through a filter that does not absorb levothyroxine. Transfer 10.0 mL of the filtrate to a 50-mL volumetric flask, dilute with 0.01 M Methanolic sodium hydroxide to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) in the portion of Oral Powder taken by the formula:

$$(798.85 / 776.87)(1.25C)(r_U / r_S)$$

in which 798.85 and 776.87 are the molecular weights of levothyroxine sodium and levothyroxine, respectively; C is the concentration, in µg per mL, of USP Levothyroxine RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Levothyroxine Sodium Tablets

DEFINITION

Levothyroxine Sodium Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to the levothyroxine peak of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

[NOTE—Use *Sample solution 2* for Tablets labeled to meet the requirements of *Dissolution Test 3*. For all other products, use the *Sample solution*.]

Mobile phase: Mixture of acetonitrile and water (4:6) containing 0.5 mL of phosphoric acid in each L of the mixture

Solution A: Dissolve 400 mg of sodium hydroxide in 500 mL of water. Cool, and add 500 mL of methanol.

Diluent: Mixture of methanol and water (6:4), containing 0.5 mL of phosphoric acid in each L of the mixture

Levothyroxine stock solution: 0.4 mg/mL of USP Levothyroxine RS in *Solution A*

Liothyronine stock solution: 0.4 mg/mL of USP Liothyronine RS in *Solution A*. Make a 1:100 dilution of this solution using *Mobile phase*.

Standard solution: 10 µg/mL of levothyroxine from *Levothyroxine stock solution* and 0.2 µg/mL of liothyronine from *Liothyronine stock solution*, in *Mobile phase*

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to about 100 µg of levothyroxine sodium, to a centrifuge tube, add 2 glass beads, pipet 10 mL of *Mobile phase* into the tube, and mix on a vortex mixer for 3 min. Centrifuge to obtain a clear supernatant, filtering if necessary.

Sample solution 2 (For Tablets labeled to meet the requirements of Dissolution Test 3): Place the appropriate number of Tablets (see *Table 1* below) into a suitable container, add 100.0 mL of *Diluent*, and shake by mechanical means for at least 30 min, or until the Tablets are fully disintegrated. Pass through a PTFE filter of 0.45-µm pore size.

Table 1

Tablet Strength (µg/Tablet of Levothyroxine Sodium)	Number of Tablets
Less than 100	20
At least 100 but less than 200	15
200 or more	10

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; packing L10

Flow rate: 1.5 mL/min

Injection size: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between liothyronine and levothyroxine

Relative standard deviation: NMT 2.0% for the levothyroxine peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{15}H_{10}I_4NNaO_4$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response from the *Sample solution*
- r_S = peak response from the *Standard solution*
- C_S = concentration of USP Levothyroxine RS in the *Standard solution* (µg/mL)
- C_U = nominal concentration of levothyroxine sodium in the *Sample solution* (µg/mL)
- M_{r1} = molecular weight of levothyroxine sodium, 798.85
- M_{r2} = molecular weight of levothyroxine, 776.87
- Acceptance criteria**: 95.0%–105.0%

PERFORMANCE TESTS

DISSOLUTION (711)

[NOTE—All containers that are in contact with solutions containing levothyroxine sodium are to be made of glass.]

Test 1

Medium: 0.01 N hydrochloric acid containing 0.2% sodium lauryl sulfate; 500 mL

Apparatus 2: 50 rpm

Time: 45 min

Determine the amount of $C_{15}H_{10}I_4NNaO_4$ dissolved by using the following method.

Mobile phase: Methanol and 0.1% phosphoric acid (6:4)

Standard stock solution: 0.1 mg/mL of USP Levothyroxine RS in methanol

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a solution having a concentration similar to that expected in the *Sample solution*.

Sample solution: Pass a portion of the solution under test through a suitable filter. [NOTE—Before use, check the filters for absorptive loss of drug.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection size: 800 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 4.0% of levothyroxine

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the amount of $C_{15}H_{10}I_4NNaO_4$ dissolved.

Tolerances: NLT 70% (Q) of the labeled amount of $C_{15}H_{10}I_4NNaO_4$ is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Apparatus, Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed for *Test 1*.

Time: 15 min

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the amount of $C_{15}H_{10}I_4NNaO_4$ dissolved.

Tolerances: NLT 80% (Q) of the labeled amount of $C_{15}H_{10}I_4NNaO_4$ is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium, Apparatus, Time, Standard solution, and

Sample solution: Proceed as directed in *Test 1*.

[NOTE—Filter the *Standard solution* in a manner identical to the *Sample solution*.]

Determine the amount of $C_{15}H_{10}I_4NNaO_4$ dissolved by employing the following method.

Mobile phase: Acetonitrile and water (35:65) that contains 0.5 mL/L of phosphoric acid

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L10

Temperature: 30°

Flow rate: 1.5 mL/min

Injection size: 100 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 4.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount of $C_{15}H_{10}I_4NNaO_4$ dissolved.

Tolerances: NLT 80% (Q) of the labeled amount of $C_{15}H_{10}I_4NNaO_4$ is dissolved.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

[NOTE—Do not use paddle stirrers with synthetic coating.]

Medium: 0.01 N hydrochloric acid; 500 mL for Tablets labeled to contain between 25 and 175 μ g of levothyroxine sodium; and 900 mL for Tablets labeled to contain 200 or 300 μ g of levothyroxine sodium

Apparatus 2: 75 rpm

Time: 45 min

Determine the amount of $C_{15}H_{10}I_4NNaO_4$ dissolved by using the following method.

Mobile phase: Acetonitrile, water, and phosphoric acid, (500:700:2)

Standard stock solution: Transfer about 100 mg of USP Levothyroxine RS to a 100-mL volumetric flask. Add 80 mL of alcohol and 1 mL of 1 N hydrochloric acid, sonicate for 2 min, dilute with alcohol to volume, and mix.

Standard solution: Dilute the *Standard stock solution* with a mixture of alcohol and water (1:1) to obtain a solution having a concentration of 0.01 mg/mL of levothyroxine. Dilute the resulting solution with *Medium* to obtain a final concentration similar to that expected in the *Sample solution*.

Sample solution: Sample per *Dissolution* (711). Centrifuge the solution under analysis.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.0-mm × 12.5-cm; packing L7

Flow rate: 1.5 mL/min

Injection size: 500 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 4.0% of levothyroxine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount of $C_{15}H_{10}I_4NNaO_4$ dissolved.

Tolerances: NLT 80% (Q) of the labeled amount of $C_{15}H_{10}I_4NNaO_4$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES**Organic Impurities**

- **PROCEDURE: LIMIT OF LIOTHYRONINE SODIUM**

[NOTE—Use *Sample solution 2* for Tablets labeled to meet the requirements of *Dissolution Test 3*. For all other products, use the *Sample solution*.]

Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis: Calculate the percentage of $C_{15}H_{11}I_3NNaO_4$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of liothyronine from the *Sample solution*

r_S = peak response of liothyronine from the *Standard solution*

C_S = concentration of USP Liothyronine RS in the *Standard solution* (μ g/mL)

C_U = nominal concentration of levothyroxine sodium in the *Sample solution* (μ g/mL)

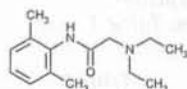
M_{r1} = molecular weight of liothyronine sodium, 672.96

M_{r2} = molecular weight of liothyronine, 650.98

Acceptance criteria: NMT 2.0% of liothyronine sodium

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
USP Levthyroxine RS
USP Liothyronine RS

Lidocaine

$C_{14}H_{22}N_2O$ 234.34
Acetamide, 2-(diethylamino)-N-(2,6-dimethylphenyl)-;
2-(Diethylamino)-2',6'-acetoxydide [137-58-6].

DEFINITION

Lidocaine contains NLT 97.5% and NMT 102.5% of $C_{14}H_{22}N_2O$.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K):** Previously dried in vacuum over silica gel for 24 h
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**PROCEDURE**

Solution A: Water and glacial acetic acid (930:50). Adjust with 1 N sodium hydroxide to a pH of 3.40.

Mobile phase: Acetonitrile and *Solution A* (1:4), so that the retention time of lidocaine is 4–6 min

Standard solution: Dissolve 85 mg of USP Lidocaine RS, with warming if necessary, in 0.5 mL of 1 N hydrochloric acid in a 50-mL volumetric flask. Dilute with *Mobile phase* to volume.

System suitability stock solution: 220 µg/mL of methylparaben in *Mobile phase*

System suitability solution: Mix 2 mL of *System suitability stock solution* and 20 mL of *Standard solution*.

Sample solution: Dissolve 85 mg of Lidocaine, with warming if necessary, in 0.5 mL of 1 N hydrochloric acid in a 50-mL volumetric flask. Dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 3.0 between lidocaine and methylparaben, *System suitability solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{14}H_{22}N_2O$ in the portion of Lidocaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)
 C_U = concentration of Lidocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.5%

IMPURITIES**Inorganic Impurities**

- **RESIDUE ON IGNITION (281):** NMT 0.1%
- **CHLORIDE AND SULFATE, Chloride (221):** Dissolve 1.0 g in a mixture of 3 mL of 2 N nitric acid and 12 mL of water, and add 1 mL of silver nitrate TS: the turbidity does not exceed that produced by 50 µL of 0.020 N hydrochloric acid (0.0035%).
- **CHLORIDE AND SULFATE, Sulfate (221):** Dissolve 100 mg in a mixture of 1 mL of 2 N nitric acid and 10 mL of water. Filter if necessary, and add 1 mL of barium chloride TS. The turbidity does not exceed that produced by 0.10 mL of 0.020 N sulfuric acid (NMT 0.1%).

Delete the following:

- **HEAVY METALS, Method I (231)**

Test preparation: 1.0 g

Analysis: Dissolve the *Test preparation* in a mixture of 2 mL of 3 N hydrochloric acid and 10 mL of water.

Evaporate on a steam bath to dryness, and dissolve the residue in 25 mL of water.

Acceptance criteria: 20 ppm • (Official 1-Jan-2018)

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE (741):** 66°–69°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Lidocaine RS

Lidocaine Topical Aerosol**DEFINITION**

Lidocaine Topical Aerosol is a solution of Lidocaine in a suitable flavored vehicle with suitable propellants in a pressurized container equipped with a metering valve. It contains NLT 90.0% and NMT 110.0% of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$), and it delivers NLT 85.0% and NMT 115.0% of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$) per actuation.

IDENTIFICATION**A.**

Sample solution: To 5 mL of Aerosol spray, collected in a separator, add 10 mL of water and 3 mL of dilute hydrochloric acid (1 in 2), wash with two 15-mL portions of chloroform, and discard the chloroform washings. Render the solution in the separator alkaline with 5–6 mL of ammonium hydroxide, and extract with three 20-mL portions of chloroform, filtering the chloroform extracts through a pledget of cotton previously moistened with chloroform. Evaporate the combined chloroform extracts with the aid of gentle heat to dryness, and dry the residue under vacuum over silica gel for 24 h.

Acceptance criteria: A potassium bromide dispersion of the lidocaine exhibits maxima only at the same wavelengths as that of a similar preparation of USP Lidocaine RS.

- **B.**
Analysis: To 2 mL of Aerosol spray, collected in a test tube, add 10–15 drops of cobaltous chloride TS, and shake for 2 min.
Acceptance criteria: A bright green color develops, and a fine precipitate is formed (lidocaine).
- **C.**
Analysis: To 2 mL of Aerosol spray, collected in a test tube, add 5 mL of water, 1 mL of 2 N nitric acid, and 3 mL of mercuric nitrate TS.
Acceptance criteria: A light yellow color develops (lidocaine).

ASSAY• **PROCEDURE**

Sample solution: Weigh 1 Aerosol container and actuator. Transfer a counted number of NLT 10 doses to a 125-mL conical flask by carefully discharging the doses in such a manner as to avoid loss of material, and take precautions to protect the sample from absorption of atmospheric moisture. Weigh the container and actuator to obtain the sample weight. To the specimen, add 20 mL of chloroform, mix, and add 10 mL of dioxane and 2 drops of crystal violet TS.

Analysis: Titrate with 0.1 N perchloric acid in dioxane VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 23.43 mg of lidocaine ($C_{14}H_{22}N_2O$).

Acceptance criteria: It contains 90.0%–110.0% of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$), and it delivers 85.0%–115.0% of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$) per actuation.

PERFORMANCE TESTS**Change to read:**

- **INHALATION AND NASAL DRUG PRODUCTS: AEROSOLS, SPRAYS, AND POWDERS—PERFORMANCE QUALITY TESTS (601) and TOPICAL AEROSOLS (603)** (CN 1-May-2017)
For Delivered-Dose Uniformity and Number of Discharges per Container
Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in nonreactive aerosol containers equipped with metered-dose valves.
- **USP REFERENCE STANDARDS (11)**
 USP Lidocaine RS

Lidocaine Ointment**DEFINITION**

Lidocaine Ointment is Lidocaine in a suitable hydrophilic ointment base. It contains NLT 95.0% and NMT 105.0% of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$).

IDENTIFICATION• **A. INFRARED ABSORPTION (197K)**

Sample: Stir a quantity of Ointment, equivalent to 300 mg of lidocaine, with 20 mL of water, transfer to a

separator, and extract with two 30-mL portions of solvent hexane. Wash the combined hexane extracts with 10 mL of water, evaporate with the aid of a current of warm air, and dry the residue in vacuum over silica gel for 24 h.

Acceptance criteria: The crystalline precipitate so obtained meets the requirements.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: 0.1% phosphoric acid, prepared by adding 1.0 mL of 85% phosphoric acid to 1 L of water

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	10	90
10.1	90	10
15	90	10

Diluent: Acetonitrile and *Solution A* (1:1)

System suitability solution: 0.1 mg/mL of USP Lidocaine RS and 0.04 mg/mL of USP Ropivacaine Related Compound A RS in *Diluent*

[NOTE—USP Ropivacaine Related Compound A RS is 2,6-dimethylaniline hydrochloride.]

Standard solution: 0.1 mg/mL of USP Lidocaine RS in *Diluent*

Sample solution: Nominally 0.1 mg/mL of lidocaine in *Diluent* from a portion of Ointment. Sonicate the solution for about 5 min.

Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 0.8 mL/min

Injection volume: 5 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 2,6-dimethylaniline and lidocaine are about 0.93 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 1.5 for the lidocaine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Resolution: NLT 1.8 between lidocaine and 2,6-dimethylaniline, *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
- r_S = peak response from the *Standard solution*
- C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)
- C_U = nominal concentration of lidocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **MINIMUM FILL** (755): Meets the requirements

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
USP Lidocaine RS
USP Ropivacaine Related Compound A RS
2,6-Dimethylaniline hydrochloride.
 $C_8H_{12}ClN$ 157.64

Lidocaine Oral Topical Solution

» Lidocaine Oral Topical Solution contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$). It contains a suitable flavor.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Lidocaine RS

Identification—Transfer a quantity of Oral Topical Solution, equivalent to about 250 mg of lidocaine, to a separator with 20 mL of water, and extract with 20 mL of chloroform. Wash the chloroform extract with 20 mL of water, and evaporate the chloroform extract with the aid of a current of warm air. Dissolve the residue in hexane, evaporate with the aid of a current of warm air, and dry the residue in vacuum over silica gel for 24 hours: the crystalline precipitate so obtained responds to *Identification test A* under *Lidocaine*.

Assay—Transfer an accurately measured volume of Oral Topical Solution, equivalent to about 150 mg of lidocaine, to a 125-mL conical flask, and protect from atmospheric moisture with a stopper fitted with a tube containing silica gel. Add 20 mL of glacial acetic acid and 2 drops of crystal violet TS. Titrate immediately with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 23.43 mg of $C_{14}H_{22}N_2O$.

Lidocaine Hydrochloride

$C_{14}H_{22}N_2O \cdot HCl \cdot H_2O$	288.81
Acetamide, 2-(diethylamino)-N-(2,6-dimethylphenyl)-, monohydrochloride, monohydrate;	
2-(Diethylamino)-2',6'-acetoxydide monohydrochloride monohydrate [6108-05-0].	
Anhydrous	270.80
[73-78-9].	

DEFINITION

Lidocaine Hydrochloride contains NLT 97.5% and NMT 102.5% of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements

ASSAY

PROCEDURE

Solution A: Water and glacial acetic acid (930:50). Adjust with 1 N sodium hydroxide to a pH of 3.40.

Mobile phase: Acetonitrile and *Solution A* (1:4)

Standard solution: 2.0 mg/mL of USP Lidocaine Hydrochloride RS in *Mobile phase*

System suitability stock solution: 220 µg/mL of methylparaben in *Mobile phase*

System suitability solution: Mix 2 mL of *System suitability stock solution* and 20 mL of *Standard solution*.

Sample solution: 2 mg/mL of Lidocaine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 3.0 between lidocaine and methylparaben, *System suitability solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) in the portion of Lidocaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lidocaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Lidocaine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.5% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

- **CHLORIDE AND SULFATE, Sulfate** (221)

Sample: 100 mg

Analysis: Dissolve *Sample* in 10 mL of water, and add 1 mL of 3 N hydrochloric acid. Mix, and add 1 mL of barium chloride TS.

Acceptance criteria: The turbidity does not exceed that produced by 0.10 mL of 0.020 N sulfuric acid (NMT 0.1%).

Delete the following:

- **HEAVY METALS, Method I** (231): NMT 20 ppm (Official 1-Jan-2018)

- **ORGANIC IMPURITIES**

Buffer: 4.85 g/L of monobasic potassium phosphate in water. Adjust with sodium hydroxide solution to a pH of 8.0.

Mobile phase: Acetonitrile and *Buffer* (30:70)

Standard solution: 0.5 µg/mL of USP Ropivacaine Related Compound A RS and 5 µg/mL each of USP Lido-

caïne Related Compound H RS and USP Lidocaine Hydrochloride RS in *Mobile phase*

Sample solution: 5 mg/mL of Lidocaine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 3.9-mm × 15-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: NLT 3.5 times the retention time for lidocaine

System suitability

Sample: *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between lidocaine related compound H and ropivacaine related compound A; NLT 2.0 between ropivacaine related compound A and lidocaine

Relative standard deviation: NMT 10.0% for ropivacaine related compound A

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lidocaine related compound H or ropivacaine related compound A in the portion of Lidocaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for lidocaine related compound H or ropivacaine related compound A from the *Sample solution*

r_S = peak response of lidocaine related compound H or ropivacaine related compound A from the *Standard solution*

C_S = concentration of USP Lidocaine Related Compound H RS or USP Ropivacaine Related Compound A RS in the *Standard solution* (μg/mL)

C_U = concentration of Lidocaine Hydrochloride in the *Sample solution* (μg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Lidocaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each unspecified impurity from the *Sample solution*

r_S = peak response of lidocaine from the *Standard solution*

C_S = concentration of USP Lidocaine Hydrochloride RS in the *Standard solution* (μg/mL)

C_U = concentration of Lidocaine Hydrochloride in the *Sample solution* (μg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lidocaine related compound H	0.38	0.10
Ropivacaine related compound A	0.42	0.01
Lidocaine	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS

• **WATER DETERMINATION, Method I (921):** 5.0%–7.0%

• **STERILITY TESTS (71):** Where the label states that Lidocaine Hydrochloride is sterile, it meets the requirements.

• **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Lidocaine Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 1.1 USP Endotoxin Units/mg of lidocaine hydrochloride.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP REFERENCE STANDARDS (11)**

USP Endotoxin RS

USP Lidocaine Hydrochloride RS

USP Lidocaine Related Compound H RS

2-Chloro-N-(2,6-dimethylphenyl)acetamide.

$C_{10}H_{12}ClNO$ 197.66

USP Ropivacaine Related Compound A RS

2,6-Dimethylaniline hydrochloride.

$C_8H_{11}N \cdot HCl$ 157.64

Lidocaine Hydrochloride Injection

» Lidocaine Hydrochloride Injection is a sterile solution of Lidocaine Hydrochloride in Water for Injection, or a sterile solution prepared from Lidocaine with the aid of Hydrochloric Acid in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass. Injection may be packaged in 50-mL multiple-dose containers.

Labeling—Injections that are of such concentration that they are not intended for direct injection into tissues are labeled to indicate that they are to be diluted prior to administration.

USP Reference standards (11)—

USP Endotoxin RS

USP Lidocaine RS

Identification—Place in a separator a volume of Injection equivalent to about 300 mg of lidocaine hydrochloride, and extract with four 15-mL portions of chloroform, discarding the chloroform extracts. Add 2 mL of 2 N sodium hydroxide to the aqueous solution remaining in the separator, and extract with four 15-mL portions of chloroform. Combine the chloroform extracts, and evaporate with the aid of a current of warm air to dryness. Dissolve the crystals so obtained in solvent hexane, evaporate with the aid of warm air, and dry the residue in vacuum over silica gel for 24 hours: the residue so obtained responds to *Identification test A* under *Lidocaine*.

Bacterial Endotoxins Test (85)—It contains not more than 1.1 USP Endotoxin Units per mg of lidocaine hydrochloride.

pH (791): between 5.0 and 7.0.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products (1)*.

Assay—Proceed with Injection as directed in the Assay for lidocaine hydrochloride under Lidocaine Hydrochloride and Epi-nephine Injection.

Lidocaine Hydrochloride Jelly

DEFINITION

Lidocaine Hydrochloride Jelly is Lidocaine Hydrochloride in a suitable, water-soluble, sterile, viscous base. It contains NLT 95.0% and NMT 105.0% of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

Standard: Prepare as directed in *Infrared Absorption* (197K), using USP Lidocaine RS.

Sample: Place in a separator containing 10–15 mL of water a quantity of Jelly, equivalent to 300 mg of lidocaine hydrochloride. Mix to assure thorough dilution of the Jelly, and add 4 mL of 6 N ammonium hydroxide. Extract with four 15-mL portions of chloroform. Combine the chloroform extracts, and evaporate with the aid of a current of warm air to dryness. Redissolve the crystals in solvent hexane, evaporate with the aid of warm air, and dry the residue in vacuum over silica gel for 24 h.

Acceptance criteria: The residue so obtained meets the requirements.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: 0.1% phosphoric acid, prepared by adding 1.0 mL of 85% phosphoric acid to 1 L of water

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	10	90
10.1	90	10
15	90	10

Diluent: Acetonitrile and *Solution A* (1:1)

System suitability solution: 0.1 mg/mL of USP Lidocaine RS and 0.04 mg/mL of USP Ropivacaine Related Compound A RS in *Diluent*

[NOTE—USP Ropivacaine Related Compound A RS is 2,6-dimethylaniline hydrochloride.]

Standard solution: 0.1 mg/mL of USP Lidocaine RS in *Diluent*

Sample solution: Nominally 0.12 mg/mL of lidocaine hydrochloride in *Diluent* from a portion of Jelly. Sonicate the solution for about 10 min.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 0.8 mL/min

Injection volume: 5 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times of 2,6-dimethylaniline and lidocaine are about 0.93 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 1.5 for the lidocaine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Resolution: NLT 1.8 between lidocaine and 2,6-dimethylaniline, *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) in the portion of Jelly taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of lidocaine hydrochloride, 270.80

M_{r2} = molecular weight of lidocaine, 234.34

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

SPECIFIC TESTS

- **pH (791):** 6.0–7.0

- **STERILITY TESTS (71):** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

- **USP REFERENCE STANDARDS (11)**

USP Lidocaine RS

USP Ropivacaine Related Compound A RS

2,6-Dimethylaniline hydrochloride.

$C_8H_{12}ClN$ 157.64

Lidocaine Hydrochloride Oral Topical Solution

DEFINITION

Lidocaine Hydrochloride Oral Topical Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$). It contains a suitable flavor and/or sweetening agent.

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

Sample: Place in a separator a volume of Oral Topical Solution, equivalent to 300 mg of lidocaine hydrochloride, and extract with four 15-mL portions of chloroform, discarding the chloroform extracts. Add 2 mL of 2 N sodium hydroxide to the aqueous solution remaining in the separator, and extract with four 15-mL portions of chloroform. Combine the chloroform extracts, and evaporate with the aid of a current of warm air to dryness. Dissolve the crystals in solvent hexane, evaporate with the aid of warm air, and dry the residue under vacuum over silica gel for 24 h.

Acceptance criteria: Residue obtained from the *Sample* meets the requirements.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY**• PROCEDURE**

Solution A: 4.85 g/L of monobasic potassium phosphate. Adjust with 10 N sodium hydroxide solution to a pH of 8.00.

Mobile phase: Acetonitrile and *Solution A* (30:70)

System suitability stock solution: 0.043 mg/mL of USP Lidocaine RS (equivalent to 0.05 mg of lidocaine hydrochloride), 0.05 mg/mL of USP Lidocaine Related Compound H RS, and 0.0065 mg/mL of USP Ropivacaine Related Compound A RS in *Mobile phase* prepared as follows. Transfer a weighed quantity of USP Lidocaine RS, USP Lidocaine Related Compound H RS, and USP Ropivacaine Related Compound A RS to a suitable volumetric flask, and add a small amount of acetonitrile. Swirl to dissolve, and dilute with *Mobile phase* to volume.

System suitability solution: Transfer 2.0 mL of *System suitability stock solution* to a 20-mL volumetric flask, and dilute with *Mobile phase* to volume.

Standard solution: 0.85 mg/mL of USP Lidocaine RS (equivalent to 1 mg/mL of lidocaine hydrochloride) in *Mobile phase*

Sample solution: Nominally equivalent to 1 mg/mL of lidocaine hydrochloride from Oral Topical Solution in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between lidocaine related compound H and ropivacaine related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) in the portion of Oral Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of lidocaine from the *Sample solution*

r_S = peak response of lidocaine from the *Standard solution*

C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of lidocaine hydrochloride, 270.80

M_{r2} = molecular weight of lidocaine, 234.34

Acceptance criteria: 95.0%–105.0%

RS, and 0.00065 mg/mL of USP Ropivacaine Related Compound A RS in *Mobile phase*

Sample solution: Nominally equivalent to 5 mg/mL of lidocaine hydrochloride in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between lidocaine related compound H and ropivacaine related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0% for lidocaine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lidocaine related compound H in the portion of Oral Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lidocaine related compound H from the *Sample solution*

r_S = peak response of lidocaine related compound H from the *Standard solution*

C_S = concentration of USP Lidocaine Related Compound H RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of dimethylaniline in the portion of Oral Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of dimethylaniline from the *Sample solution*

r_S = peak response of dimethylaniline from the *Standard solution*

C_S = concentration of USP Ropivacaine Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lidocaine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of ropivacaine related compound A, 157.64

M_{r2} = molecular weight of dimethylaniline, 121.18

Calculate the percentage of any other individual impurity in the portion of Oral Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of lidocaine from the *Standard solution*

C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of lidocaine hydrochloride, 270.80

M_{r2} = molecular weight of lidocaine, 234.34

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lidocaine related compound H	0.33	0.1
Dimethylaniline	0.37	0.01

IMPURITIES**• ORGANIC IMPURITIES**

Solution A, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.0043 mg/mL of USP Lidocaine RS, 0.005 mg/mL of USP Lidocaine Related Compound H

Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lidocaine	1.0	—
Any other individual, unspecified impurity	—	0.10
Total impurities	—	—

SPECIFIC TESTS

- **pH** (791): 5.0–7.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
 - USP Lidocaine RS
 - USP Lidocaine Related Compound H RS
 - N-(Chloroacetyl)-2,6-xylidide.
 - $C_{10}H_{12}ClNO$ 197.66
 - USP Ropivacaine Related Compound A RS
 - 2,6-Dimethylaniline hydrochloride.
 - $C_8H_{11}N \cdot HCl$ 157.64

Lidocaine Hydrochloride Topical Solution

DEFINITION

Lidocaine Hydrochloride Topical Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

Sample: Place in a separator a volume of Topical Solution, equivalent to 200 mg of lidocaine hydrochloride, and extract with four 15-mL portions of chloroform, discarding the chloroform extracts. Add 2 mL of 2 N sodium hydroxide to the aqueous solution remaining in the separator, and extract with four 15-mL portions of chloroform. Combine the chloroform extracts, and evaporate with the aid of a current of warm air to dryness. Dissolve the crystals in solvent hexane, evaporate with the aid of warm air, and dry the residue under vacuum over silica gel for 24 h.

Acceptance criteria: The residue obtained from the *Sample* meets the requirements.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

- **PROCEDURE**

Solution A: Glacial acetic acid and water (5:93). Adjust with 1 N sodium hydroxide to a pH of 3.40.

Mobile phase: Acetonitrile and *Solution A* (1:4)

Standard solution: 1.7 mg/mL of USP Lidocaine RS (equivalent to 2 mg/mL of lidocaine hydrochloride) in *Mobile phase* prepared as follows. Transfer a weighed quantity of USP Lidocaine RS to a suitable volumetric flask, and add 1 N hydrochloric acid to fill 1% of the final volume. Warm if necessary, and dilute with *Mobile phase* to volume.

System suitability stock solution: 220 µg/mL of USP Methylparaben RS in *Mobile phase*

System suitability solution: Mix 2 mL of the *System suitability stock solution* with 20 mL of the *Standard solution*.

Sample solution: Nominally equivalent to 2 mg/mL of lidocaine hydrochloride from Topical Solution in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 3.0 between lidocaine and methylparaben, *System suitability solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of lidocaine from the *Sample solution*

r_S = peak response of lidocaine from the *Standard solution*

C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of lidocaine hydrochloride, 270.80

M_{r2} = molecular weight of lidocaine, 234.34

Acceptance criteria: 95.0%–105.0%

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A and Mobile phase: Proceed as directed in the Assay.

System suitability solution: 2.6 µg/mL of USP Lidocaine RS, 3.9 µg/mL of USP Ropivacaine Related Compound A RS, and 3 µg/mL of USP Lidocaine Related Compound H RS in *Mobile phase*

Standard solution: 0.0017 mg/mL of USP Lidocaine RS, 0.0026 mg/mL of USP Ropivacaine Related Compound A RS (equivalent to 0.002 mg/mL of 2,6-dimethylaniline), and 0.002 mg/mL of USP Lidocaine Related Compound H RS in *Mobile phase*

Sample solution: Nominally equivalent to 2 mg/mL of lidocaine hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See Table 1 for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between lidocaine related compound H and ropivacaine related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0% for lidocaine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lidocaine related compound H in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of lidocaine related compound H from the *Sample solution*
 r_S = peak response of lidocaine related compound H from the *Standard solution*
 C_S = concentration of USP Lidocaine Related Compound H RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of dimethylaniline in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response of dimethylaniline from the *Sample solution*
 r_S = peak response of dimethylaniline from the *Standard solution*
 C_S = concentration of USP Ropivacaine Related Compound A RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of ropivacaine related compound A, 157.64
 M_{r2} = molecular weight of dimethylaniline, 121.84
 Calculate the percentage of any impurity in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response of each impurity from the *Sample solution*
 r_S = peak response of lidocaine from the *Standard solution*
 C_S = concentration of USP Lidocaine RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of lidocaine hydrochloride in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of lidocaine hydrochloride, 270.80
 M_{r2} = molecular weight of lidocaine, 234.34

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lidocaine	1.0	—
Dimethylaniline	3.2	0.1
Lidocaine related compound H	3.8	0.1

Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any unspecified impurity	—	0.10
Total impurities	—	2.0

SPECIFIC TESTS

- pH (791):** 5.0–7.0

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
 - USP Lidocaine RS
 - USP Lidocaine Related Compound H RS
 - N-(Chloroacetyl)-2,6-xylylidide.
 - $C_{10}H_{12}ClNO$ 197.66
 - USP Methylparaben RS
 - USP Ropivacaine Related Compound A RS
 - 2,6-Dimethylaniline hydrochloride.
 - $C_8H_{11}N \cdot HCl$ 157.64

Lidocaine Hydrochloride and Dextrose Injection

» Lidocaine Hydrochloride and Dextrose Injection is a sterile solution of Lidocaine Hydrochloride and Dextrose in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) and dextrose ($C_6H_{12}O_6 \cdot H_2O$).

Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

USP Reference standards (11)—

- USP Endotoxin RS
- USP Lidocaine RS

Identification—

A: Place in a separator a volume of Injection equivalent to about 300 mg of lidocaine hydrochloride, add 2 mL of 2 N sodium hydroxide, and extract with four 15-mL portions of chloroform. Combine the chloroform extracts, and evaporate with the aid of a current of warm air to dryness. Dissolve the crystals so obtained in solvent hexane, evaporate with the aid of warm air, and dry the residue in vacuum over silica gel for 24 hours; the residue so obtained responds to *Identification test A* under *Lidocaine*.

B: It responds to the *Identification test* under *Dextrose*.

Bacterial Endotoxins Test (85)—It contains not more than 1.1 USP Endotoxin Units per mg of lidocaine hydrochloride.

pH (791): between 3.0 and 7.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products (1)*.

Assay for lidocaine hydrochloride—Proceed with Injection as directed in the *Assay for lidocaine hydrochloride* under *Lidocaine and Epinephrine Injection*.

Assay for dextrose—Determine the angular rotation of Injection in a suitable polarimeter tube (see *Optical Rotation (781)*). Calculate the percentage (g per 100 mL) of dextrose

($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively; A is 100 mm divided by the length of the polarimeter tube, in mm; and R is the observed rotation, in degrees.

Lidocaine Hydrochloride and Epinephrine Injection

» Lidocaine Hydrochloride and Epinephrine Injection is a sterile solution prepared from Lidocaine Hydrochloride and Epinephrine with the aid of Hydrochloric Acid in Water for Injection, or a sterile solution prepared from Lidocaine and Epinephrine with the aid of Hydrochloric Acid in Water for Injection, or a sterile solution of Lidocaine Hydrochloride and Epinephrine Bitartrate in Water for Injection. The content of epinephrine does not exceed 0.002 percent (1 in 50,000). Lidocaine Hydrochloride and Epinephrine Injection contains the equivalent of not less than 95.0 percent and not more than 105.0 percent of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) and the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of epinephrine ($C_9H_{13}NO_3$).

Packaging and storage—Preserve in single-dose or multiple-dose light-resistant containers, preferably of Type I glass.

Labeling—The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

USP Reference standards (11)—

USP Endotoxin RS

USP Epinephrine Bitartrate RS

USP Lidocaine RS

Color and clarity—Using the Injection as the *Test solution*, proceed as directed for *Color and clarity* under *Epinephrine Injection*.

Bacterial Endotoxins Test (85)—It contains not more than 0.7 USP Endotoxin Unit per mg of lidocaine hydrochloride.

pH (791): between 3.3 and 5.5.

Other requirements—It responds to the *Identification* test under *Lidocaine Hydrochloride Injection*. It meets also the requirements under *Injections and Implanted Drug Products* (1).

Assay for lidocaine hydrochloride—

Mobile phase—Mix 50 mL of glacial acetic acid and 930 mL of water, and adjust with 1 N sodium hydroxide to a pH of 3.40. Mix about 4 volumes of this solution with 1 volume of acetonitrile, so that the retention time of lidocaine is about 4 to 6 minutes. Pass through a membrane filter having a 1- μ m or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve about 85 mg of USP Lidocaine RS, accurately weighed, with warming if necessary, in 0.5 mL of 1 N hydrochloric acid in a 50-mL volumetric flask,

dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 1.7 mg of lidocaine per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of lidocaine hydrochloride, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Resolution preparation—Prepare a solution of methylparaben in *Mobile phase* containing about 220 μ g per mL. Mix 2 mL of this solution and 20 mL of the *Standard preparation*.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm \times 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph about 20 μ L of the *Resolution preparation*, and record the peak responses as directed for *Procedure*: the resolution, R , between lidocaine and methylparaben is not less than 3.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Assay preparation* and the *Standard preparation* into the chromatograph. Record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) in each mL of the Injection taken by the formula:

$$(270.80/234.34)(50)(C/V)(r_U/r_S)$$

in which 270.80 and 234.34 are the molecular weights of lidocaine hydrochloride and lidocaine, respectively; C is the concentration, in mg per mL, of USP Lidocaine RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and r_U and r_S are the lidocaine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for epinephrine—

Mobile phase—Mix 50 mL of glacial acetic acid and 930 mL of water, and adjust with 1 N sodium hydroxide to a pH of 3.40. Dissolve 1.1 g of sodium 1-heptanesulfonate in this solution, add 1.0 mL of 0.1 M edetate disodium, and mix. Mix about 9 volumes of this solution with 1 volume of methanol, so that the retention time of epinephrine is about 4 to 6 minutes. Pass through a membrane filter having a 1- μ m or finer porosity, and degas.

Standard preparation—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in *Mobile phase* to obtain a solution having a known concentration of about 9 μ g of epinephrine bitartrate per mL. Pipet 10 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 1.8 μ g of epinephrine bitartrate per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 μ g of epinephrine, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is fitted with a 3.9-mm \times 30-cm stainless steel column that contains packing L1 and is equipped with an electrochemical detector held at a potential of +650 mV, a controller capable of regulating the background current, and a suitable recorder. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation* as directed for *Procedure*: the relative standard deviation of the peak responses of successive injections of the *Standard preparation* is not more than 1.5%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Assay preparation* and the *Standard preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, adjusting the specimen size and other operating parameters so that satisfactory chromatography and

peak responses are obtained. Record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in μg , of epinephrine ($\text{C}_9\text{H}_{13}\text{NO}_3$) in each mL of the Injection taken by the formula:

$$(183.20/333.29)(50)(C/V)(r_U/r_S)$$

in which 183.20 and 333.29 are the molecular weights of epinephrine and epinephrine bitartrate, respectively; C is the concentration, in μg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Lidocaine and Prilocaine Cream

» Lidocaine and Prilocaine Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of lidocaine ($\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}$) and prilocaine ($\text{C}_{13}\text{H}_{20}\text{N}_2\text{O}$).

Packaging and storage—Preserve in collapsible tubes or in tight containers. Do not store above 30° . Do not freeze.

USP Reference standards (11)—

USP Lidocaine RS

USP Prilocaine Hydrochloride RS

USP Prilocaine Related Compound B RS

(RS)-N-(4-Methylphenyl)-2-(propylamino)propanamide.
 $\text{C}_{13}\text{H}_{20}\text{N}_2\text{O}$ 220.31

Identification—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The total aerobic microbial count does not exceed 100 cfu per g, and the total combined molds and yeasts count does not exceed 50 cfu per g.

Minimum fill (755): meets the requirements.

pH (791): between 8.7 and 9.7, determined in a solution (1 in 10) or in the undiluted Cream.

Related compounds—

Solution A, *Solution B*, *Mobile phase*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

Standard solution—Dissolve accurately weighed quantities of USP Lidocaine RS and USP Prilocaine Hydrochloride RS in *Solution A*, and dilute quantitatively, and stepwise if necessary, with *Solution A* to obtain a solution having a known concentration of about 0.002 mg per mL of each compound. Immediately store this solution at or below 10° .

Test solution—Use the *Assay preparation*, prepared as directed in the *Assay*.

Chromatographic system (see Chromatography (621))—Proceed as directed in the *Assay*. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are listed in *Table 1*; and the resolution, R , between prilocaine and prilocaine related compound B is not less than 1.4. Chromatograph the *Standard solution* a minimum of six times, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 50 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each related compound in the portion of the Cream taken by the formula:

$$100C(r_U/r_S)(V/W)(100/L)(1/F)(220.31/256.77)$$

in which C is the individual concentration, in mg per mL, of either USP Lidocaine RS or USP Prilocaine Hydrochloride RS in the *Standard solution*; r_U is the individual peak response of the impurities obtained from the *Test solution*; r_S is the individual peak response for either lidocaine or prilocaine obtained from the *Standard solution*; V is the volume, in mL, of the *Test solution*; W is the weight, in mg, of the Cream taken to prepare the *Test solution*; L is the individual label claim, in percent, for either lidocaine or prilocaine; F is the relative response factor for each related compound as listed in *Table 1*; and 220.31 and 256.77 are the molecular weights of prilocaine and prilocaine hydrochloride, respectively (these are used only for calculation involving prilocaine related compounds). The percentages of lidocaine related compounds and prilocaine related compounds are calculated using the concentration and peak response from USP Lidocaine RS and USP Prilocaine Hydrochloride RS, respectively. The designation of whether an impurity is a lidocaine related compound or prilocaine related compound is specified in *Table 1*. The percentage of any individual unknown related compound is determined using the concentration and peak response from USP Prilocaine Hydrochloride RS in the *Standard solution*.

Table 1

Related Compound	Relative Retention Time ^a	Relative Response Factor (F)	Limit
o-Toluidine	0.38	2.3 (P) ^b	not more than 2.0%
n-Chloroacetyl-2,6-xyldine	0.54	1.0 (L) ^c	not more than 0.1%
2,6-Dimethylaniline	0.67	3.3 (L) ^c	not more than 0.1%
Prilocaine	1.00	—	—
2-Diethylaminoaceto-2,4-xyldine	1.33	0.8 (L) ^c	not more than 0.1%
Lidocaine	2.14	—	—
n-Dichloroacetyl-2,6-xyldine	2.98	2.2 (L) ^c	not more than 0.1%
Any other individual related compounds	—	1.0 (P) ^b	not more than 0.2%
Total related compounds, excluding o-toluidine	—	—	not more than 1.0%

^a Relative to the prilocaine peak.

^b P designates a prilocaine related compound.

^c L designates a lidocaine related compound.

Assay—

Solution A—Dissolve about 2.73 g of monobasic potassium phosphate in 630 mL of water, and adjust with 5 N sodium hydroxide to a pH of 7.20 ± 0.02 . Dilute with acetonitrile to 1 L.

Solution B—Dissolve about 2.73 g of monobasic potassium phosphate in 900 mL of water, and adjust with 5 N sodium hydroxide to a pH of 7.20 ± 0.02 . Dilute with acetonitrile to 1 L.

Mobile phase—Use variable mixtures of filtered and degassed **Solution A** and **Solution B** as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

Standard preparation—Dissolve accurately weighed quantities of USP Lidocaine RS and USP Prilocaine Hydrochloride RS in **Solution A**, and dilute quantitatively, and stepwise if necessary, with **Solution A** to obtain a solution having a known concentration of about 0.2 mg per mL of each compound. Immediately store this solution at or below 10°.

System suitability solution—Dissolve an accurately weighed quantity of USP Prilocaine Related Compound B RS in the **Standard preparation**, and dilute quantitatively, and stepwise if necessary, with the **Standard preparation**, to obtain a solution having a known concentration of about 0.08 mg per mL of prilocaine related compound B.

Assay preparation—Transfer a portion of the Cream, equivalent to about 20 mg each of lidocaine and prilocaine, accurately weighed, to a 100-mL volumetric flask. Add 5 mL of 5 N sodium hydroxide to disperse the Cream, and mix. Add 5 mL of 5 N hydrochloric acid, and dilute with **Solution A** to volume, and mix. Pass a portion through a nylon filter having a 0.2-μm or finer porosity, discarding the first 1 mL, and use the filtrate. Immediately store this solution at or below 10°.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 232-nm detector and a 4.6-mm × 10-cm column that contains 3-μm packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 40°. The samples are maintained at or below 10°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	67	33	equilibration
0–11.0	67	33	isocratic
11.0–22.0	67→100	33→0	linear gradient
22.0–32.0	100	0	isocratic

Chromatograph the **System suitability solution**, and record the peak responses as directed for *Procedure*: the relative retention times are 1.00 for prilocaine, 1.09 for prilocaine related compound B, and 2.14 for lidocaine; and the resolution, R , between prilocaine and prilocaine related compound B is not less than 1.4. Chromatograph the **Standard preparation** a minimum of five times, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 5000 theoretical plates, based on the prilocaine peak; the tailing factor is not more than 1.5, based on the prilocaine peak; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 μL) of the **Standard preparation** and the **Assay preparation** into the chromatograph, record the chromatograms, and measure the responses for the lidocaine and prilocaine peaks. Calculate the percentage of the label claim of lidocaine

($C_{14}H_{22}N_2O$) and prilocaine ($C_{13}H_{20}N_2O$) in the portion of Cream taken by the formula:

$$100C(r_U / r_S)(V/W)(100/L)(220.31/256.77)$$

in which C is the individual concentration, in mg per mL, of either USP Lidocaine RS or USP Prilocaine Hydrochloride RS in the **Standard preparation**; r_U and r_S are either the individual peak responses of lidocaine or prilocaine obtained from the **Assay preparation** and the **Standard preparation**, respectively; V is the volume, in mL, of the **Assay preparation**; W is the weight, in mg, of the Cream taken to prepare the **Assay preparation**; L is the individual label claim, in percent, for either lidocaine or prilocaine; and 220.31 and 256.77 are the molecular weights of prilocaine and prilocaine hydrochloride, respectively (these are used only for calculating the percentage of prilocaine in the Cream).

Lime

CaO 56.08

Calcium oxide [1305-78-8].

DEFINITION

Lime, when freshly ignited to constant weight, contains NLT 95.0% of lime (CaO).

IDENTIFICATION

- **A.** **Analysis:** Moisten a suitable quantity of Lime with water: heat is generated, and a white powder is obtained (calcium hydroxide or slaked lime). Mix the powder with 3 or 4 times its weight of water.
Acceptance criteria: A smooth magma of lime forms that is alkaline to litmus.
- **B. IDENTIFICATION TESTS—GENERAL, Calcium (191)**
Sample solution: Slake 1 g with 20 mL of water, and add 6 N acetic acid until the lime is dissolved.
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
Sample solution: Ignite 1 g of Lime in a muffle furnace to constant weight. Cool, weigh accurately, and dissolve in 20 mL of 3 N hydrochloric acid. Cool the solution, transfer to a 500-mL volumetric flask with the aid of water, and dilute with water to volume.
Analysis: Transfer 50.0 mL to a suitable container, add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue in color. Each mL of 0.05 M edetate disodium is equivalent to 2.804 mg of lime (CaO).
Acceptance criteria: NLT 95.0%

IMPURITIES

- **INSOLUBLE SUBSTANCES**
Sample: 5.0 g
Analysis: Slake the **Sample**, then mix with 100 mL of water, followed by hydrochloric acid, dropwise, with agitation, until solution takes place: the resulting solution after boiling and cooling is acid. Filter the solution through a tared crucible, wash with water until free of chlorides, and dry at 105° for 1 h.
Acceptance criteria: NMT 50 mg (1.0%) of insoluble substances
- **MAGNESIUM AND ALKALI SALTS**
Sample solution: Dissolve 500 mg in 30 mL of water and 15 mL of 3 N hydrochloric acid. Neutralize the solution with 6 N ammonium hydroxide, heat to boiling, and add ammonium oxalate TS to precipitate the cal-

cium completely. Heat the mixture on a steam bath for 1 h. Cool, dilute with water to 100 mL, mix, and filter.

Analysis: To 50 mL of the filtrate add 0.5 mL of sulfuric acid, evaporate to dryness, and ignite in a tared platinum crucible to constant weight.

Acceptance criteria: The weight of the residue does not exceed 9 mg.

• **CARBONATE**

Sample: 1 g

Analysis: Slake the *Sample*, mix with 50 mL of water, and decant the greater portion of the milky liquid.

Acceptance criteria: The addition of an excess of 3 N hydrochloric acid to the residue does not cause more than a slight effervescence.

SPECIFIC TESTS

• **LOSS ON IGNITION** (733)

Analysis: Ignite a portion to constant weight in a tared platinum crucible at $1100 \pm 50^\circ$.

Acceptance criteria: NMT 10.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

Lincomycin Injection

» Lincomycin Injection contains an amount of Lincomycin Hydrochloride in Water for Injection equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of lincomycin ($C_{18}H_{34}N_2O_6S$). It contains benzyl alcohol as a preservative.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards (11)—

USP Endotoxin RS

USP Lincomycin Hydrochloride RS

Bacterial Endotoxins Test (85)—It contains not more than 0.5 USP Endotoxin Unit per mg of lincomycin.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH (791): between 3.0 and 5.5.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

*Mobile phase, Standard preparation, and Chromatographic system—*Proceed as directed in the *Assay* under *Lincomycin Hydrochloride*.

*Assay preparation—*Transfer an accurately measured volume of Injection, equivalent to about 600 mg of lincomycin, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Procedure—*Proceed as directed for *Procedure* in the *Assay* under *Lincomycin Hydrochloride*. Calculate the quantity, in mg, of lincomycin ($C_{18}H_{34}N_2O_6S$) in each mL of the Injection taken by the formula:

$$0.625(CP/V)(r_U/r_S)$$

in which *V* is the volume, in mL, of Injection taken, and the other terms are as defined therein.

Lincomycin Oral Solution

» Lincomycin Oral Solution contains an amount of lincomycin hydrochloride ($C_{18}H_{34}N_2O_6S \cdot HCl \cdot H_2O$) equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of lincomycin ($C_{18}H_{34}N_2O_6S$), and one or more suitable colors, flavors, preservatives, and sweeteners in water.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Lincomycin Hydrochloride RS

Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698): meets the requirements.

pH (791): between 3 and 5.5.

Assay—

*Mobile phase, Standard preparation, and Chromatographic system—*Proceed as directed in the *Assay* under *Lincomycin Hydrochloride*.

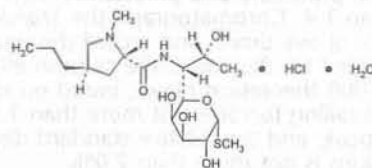
*Assay preparation—*Transfer an accurately measured volume of Oral Solution, freshly mixed and free of air bubbles, equivalent to about 100 mg of lincomycin, to a suitable container. Add 0.5 mL of sodium carbonate solution (3 in 10), and swirl for 30 seconds, noting that a precipitate forms. Add 1.0 mL of 0.2 N sodium hydroxide, swirl for 30 seconds, add 10.0 mL of chloroform, and shake by mechanical means for 10 minutes. Centrifuge, and remove the upper aqueous layer by suction. Transfer 1.0 mL of the clear chloroform layer to a suitable container, and evaporate under a stream of nitrogen to dryness. Add 10.0 mL of *Mobile phase* to the residue, and dissolve by swirling, sonicating if necessary.

*Procedure—*Proceed as directed in the *Assay* under *Lincomycin Hydrochloride*, recording the chromatogram over a period seven times the retention time of lincomycin. Calculate the quantity, in mg, of lincomycin ($C_{18}H_{34}N_2O_6S$) in each mL of the Oral Solution taken by the formula:

$$(CP/10V)(r_U/r_S)$$

in which *V* is the volume, in mL, of Oral Solution taken; and the other terms are as defined therein.

Lincomycin Hydrochloride



$C_{18}H_{34}N_2O_6S \cdot HCl \cdot H_2O$ 461.01

D-erythro- α -D-galacto-Octopyranoside, methyl 6,8-dideoxy-6-[[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-, monohydrochloride, monohydrate, (2S-trans)-.

Methyl 6,8-dideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-D-erythro- α -D-galacto-octopyranoside monohydrochloride monohydrate [7179-49-9].

Anhydrous 443.01 [859-18-7].

» Lincomycin Hydrochloride has a potency equivalent to not less than 790 μg of lincomycin ($\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_6\text{S}$) per mg.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards (11)—

USP Endotoxin RS

USP Lincomycin Hydrochloride RS

Identification, Infrared Absorption (197M).

Specific rotation (781S): between $+135^\circ$ and $+150^\circ$.

Test solution: 20 mg per mL, in water.

Crystallinity (695): meets the requirements.

pH (791): between 3.0 and 5.5, in a solution (1 in 10).

Water Determination, Method I (921): between 3.0% and 6.0%.

Limit of lincomycin B—Use the chromatogram obtained from the *Assay preparation* in the *Assay*: the area of the lincomycin B peak is not greater than 5.0% of the sum of the areas of the lincomycin B peak and the lincomycin peak.

Other requirements—Where the label states that Lincomycin Hydrochloride is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Lincomycin Injection*. Where the label states that Lincomycin Hydrochloride must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Lincomycin Injection*.

Assay—

Mobile phase—Add 13.5 mL of phosphoric acid to 1000 mL of water, and adjust with ammonium hydroxide to a pH of 6.0. Prepare a filtered and degassed mixture of this solution, acetonitrile, and methanol (780:150:150). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Lincomycin Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 1.2 mg per mL, using sonication if necessary to effect solution.

Assay preparation—To about 12 mg of Lincomycin Hydrochloride, accurately weighed, add 10.0 mL of *Mobile phase*. Shake by mechanical means for 5 minutes, and sonicate if necessary to effect solution.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm \times 25-cm column that contains 5- μm packing L7 and is maintained at a temperature of 46° . The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor for the main lincomycin peak is not more than 1.3; the column efficiency determined from the main lincomycin peak is not less than 4000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. The relative retention times are about 0.5 for lincomycin B and 1.0 for lincomycin. Calculate the quantity, in μg , of lincomycin ($\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_6\text{S}$) in each mg of the Lincomycin Hydrochloride taken by the formula:

$$10(CP / W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Lincomycin Hydrochloride RS in the *Standard preparation*; P

is the designated potency, in μg of lincomycin per mg, of USP Lincomycin Hydrochloride RS; W is the weight, in mg, of the portion of Lincomycin Hydrochloride taken to prepare the *Assay preparation*; and r_U and r_S are the lincomycin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Lincomycin Hydrochloride Capsules

» Lincomycin Hydrochloride Capsules contain an amount of $\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_6\text{S} \cdot \text{HCl} \cdot \text{H}_2\text{O}$ equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of lincomycin ($\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_6\text{S}$).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Lincomycin Hydrochloride RS

Dissolution (711)—

Medium: water; 500 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Filter a portion of about 20 mL of the solution under test. Transfer about 5 mL of the eluant into a small test tube, and add 250 μL of 0.01 M sodium sulfate internal standard solution. Evaporate until dry using a vacuum centrifuge. Add 10.0 μL of water to the precipitate and place on a vortex mixer until all solid material is dissolved. Transfer this solution to a capillary tube, place it in a Raman spectrometer, and obtain the Raman spectrum using suitable instrumental conditions. Integrate the Raman intensity, applying baseline corrections, between 660 cm^{-1} and 720 cm^{-1} . Divide this result by the integrated intensity between 966 cm^{-1} and 994 cm^{-1} . Determine the amount of $\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_6\text{S}$ dissolved in comparison with an aqueous *Standard solution* having a known concentration of USP Lincomycin Hydrochloride RS.

Tolerances—Not less than 75% (Q) of the labeled amount of $\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_6\text{S}$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Water Determination, Method I (921): not more than 7.0%.

Assay—

Mobile phase, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay* under *Lincomycin Hydrochloride*.

Assay preparation—Remove, as completely as possible, the contents of not less than 10 Capsules, taking care to prevent capsule shell fragments from being combined with the capsule contents and to remove any shell fragments from the contents. Weigh and mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 50 mg of lincomycin, to a suitable container. Add 50.0 mL of *Mobile phase*, and shake by mechanical means for 5 minutes. Use the solution thus obtained as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Lincomycin Hydrochloride*. Calculate the quantity, in mg, of lincomycin ($\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_6\text{S}$) in the portion of Capsule contents taken by the formula:

$$(CP / 20)(r_U / r_S)$$

in which the terms are as defined therein.

Lincomycin Hydrochloride Soluble Powder

» Lincomycin Hydrochloride Soluble Powder contains an amount of Lincomycin Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lincomycin ($C_{18}H_{34}N_2O_6S$).

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Lincomycin Hydrochloride RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 6.0%.

Assay—

Mobile phase and Chromatographic system—Proceed as directed in the *Assay* under *Lincomycin Hydrochloride*.

Standard preparation—Dissolve an accurately weighed quantity of USP Lincomycin Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 1.2 mg per mL.

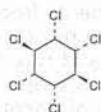
Assay preparation—Remove as completely as possible the contents of not fewer than 5 containers. Weigh and mix the combined contents, and transfer an accurately weighed portion of the Soluble Powder, equivalent to about 400 mg of lincomycin ($C_{18}H_{34}N_2O_6S$), to a 100-mL volumetric flask. Add about 80 mL of *Mobile phase*, and swirl to dissolve. Dilute with *Mobile phase* to volume, and mix. Transfer 25.0 mL of this solution to a second 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed in the *Assay* under *Lincomycin Hydrochloride*. Calculate the quantity, in mg, of lincomycin ($C_{18}H_{34}N_2O_6S$) in the portion of Soluble Powder taken by the formula:

$$0.4CP(r_U / r_S)$$

in which the terms are as defined therein.

Lindane



$C_6H_6Cl_6$ 290.83

Cyclohexane, 1,2,3,4,5,6-hexachloro-, (1 α ,2 α ,3 β ,4 α ,5 α ,6 β)- γ -1,2,3,4,5,6-Hexachlorocyclohexane [58-89-9].

» Lindane is the gamma isomer of hexachlorocyclohexane. It contains not less than 99.0 percent and not more than 101.0 percent of lindane (γ - $C_6H_6Cl_6$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Lindane RS

Identification, Infrared Absorption (197K).

Congeeing temperature (651): not less than 112.0°.

Water Determination, Method I (921): not more than 0.5%.

Chloride ion—Place about 100 mg in a test tube with 10 mL of water, shake, and filter. Add 1 mL of nitric acid and 3 mL of silver nitrate TS to the filtrate: no turbidity develops.

Assay—

Internal standard solution—Dissolve *n*-octadecane in methylene chloride to obtain a solution having a concentration of about 0.5 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Lindane RS in *Internal standard solution* to obtain a solution having a known concentration of about 2 mg per mL.

System suitability solution—Prepare solutions of α -benzene hexachlorides (BHC) at 1000 μ g per mL of methanol, β -BHC at 1000 μ g per mL of acetone, and δ -BHC at 1000 μ g per mL of methanol. Transfer 100 μ L each of α -BHC, β -BHC and δ -BHC solutions to a 4-mL conical vial, and evaporate under a stream of nitrogen to dryness. Add to the vial a 100- μ L aliquot of the *Standard preparation*. Insert the stopper, and shake vigorously to dissolve the residue.

Assay preparation—Transfer about 10 mg of Lindane, accurately weighed, to a 5-mL volumetric flask. Dissolve in and dilute with *Internal standard solution* to volume.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm \times 30-m fused-silica column coated with a 1- μ m phase G46. The chromatograph is programmed as follows. The initial column temperature is maintained at 120° for 1 minute. Then, the temperature is increased at a rate of 20° per minute to 150°, and then ramped at a rate of 10° per minute to 280° and maintained at that temperature for 4 minutes. The injection port and detector temperatures are maintained at 300°. The injection split ratio is 50:1. Chromatograph the *Standard preparation* and the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times for *n*-octadecane and lindane are about 0.85 and 1.0, respectively. [NOTE—Typical retention times for α -BHC, β -BHC, γ -BHC, δ -BHC, and *n*-octadecane are 15.7, 17.8, 16.5, 18.8, and 13.9 minutes, respectively.] The resolution, *R*, between *n*-octadecane and α -BHC is not less than 21, between lindane (γ -BHC) and α -BHC is not less than 9, between β -BHC and lindane is not less than 14, and between δ -BHC and β -BHC is not less than 8; the tailing factors for *n*-octadecane and lindane are less than 1.5 and 1.2, respectively; and the relative standard deviation of the ratios of peak area responses of lindane to *n*-octadecane for replicate injections of *Standard preparation* is not more than 1.5%.

Procedure—Separately inject equal volumes (about 1 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of γ - $C_6H_6Cl_6$ in the portion of Lindane taken by the formula:

$$5C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Lindane RS in the *Standard preparation*; and *R_U* and *R_S* are the ratios of the peak responses of lindane to *n*-octadecane, obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Lindane Cream

DEFINITION

Lindane Cream is Lindane in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of lindane ($\gamma\text{-C}_6\text{H}_6\text{Cl}_6$).

IDENTIFICATION

• A.

Analysis: Wind a strip of 20-mesh copper gauze 1.5 cm wide and 5 cm long around the end of a copper wire. Heat the gauze in the nonluminous flame of a Bunsen burner until it glows without coloring the flame green. Allow the gauze to cool, and repeat the heating and cooling step several times until a thorough coating of oxide is formed. Apply a small amount of Cream to the cooled gauze, ignite, and allow to burn freely in the air. Hold the gauze in the outer edge of the burner flame at a height of 4 cm.

Acceptance criteria: A bright green color is imparted to the flame.

ASSAY

• PROCEDURE

Mobile phase: Mix 18 mL of anhydrous ethyl ether with 280 mL of chromatographic hexane.

Internal standard solution: 1 mg/mL of *n*-docosane in methylene chloride

Standard stock solution: 2 mg/mL of USP Lindane RS in methylene chloride

Standard solution: Transfer 5.0 mL of *Standard stock solution* to a graduated centrifuge tube, add 5.0 mL of *Internal standard solution*, and evaporate with the aid of gentle heat and a current of dry air to 3 mL. Avoid evaporating to dryness. If the mixture is inadvertently evaporated to dryness, discard it, and begin another *Standard solution*.

Solid support: 60- to 100-mesh magnesium silicate that has been heated previously at 300° for 2 h

Sample stock solution: Nominally 2 mg/mL of lindane from a quantity of Cream, equivalent to 10 mg of lindane, prepared as follows. Place a pledget of cotton on a removable porous plate at the base of a 25-mm × 200-mm chromatographic tube fitted with a polytef stopcock. Add 50 mL of *Mobile phase* and 10 g of *Solid support*, and stir the mixture to expel air bubbles. Add 1.5 g of anhydrous sodium sulfate to the column, and elute until the surface of the liquid is 4 cm above the *Solid support*, discarding the eluate. Transfer a portion of Cream to a 150-mL beaker, and add 10 g of *Solid support*. Mix with a spatula, adding chromatographic hexane as necessary to produce a homogeneous mixture, and continue stirring until a free-flowing powder is produced. Transfer this mixture to the chromatographic column with the aid of three 5-mL portions of *Mobile phase*, and elute the column with 225 mL of the *Mobile phase* at a flow rate of 2–3 mL/min, collecting the eluate in a 250-mL beaker. Remove the chromatographic column, add 5.0 mL of *Internal standard solution* to the eluate, and evaporate with the aid of gentle heat and a current of dry air to 5 mL.

Sample solution: Transfer the *Sample stock solution* to a graduated centrifuge tube with the aid of 1 mL of methylene chloride, and evaporate with the aid of gentle heat and a current of dry air to 3 mL. Avoid evaporating to dryness. If the mixture is inadvertently evaporated to dryness, discard it, and begin another *Sample solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 1.8-m × 2-mm glass; packed with 3% liquid phase G3 on support S1A

Temperatures

Column: 195°

Injection port: 250°

Detector: 250°

Flow rate: 40 mL/min

Injection volume: 1 μL

Carrier gas: Dry nitrogen

System suitability

Sample: *Standard solution* (6–10 replicate injections)

Suitability requirements

Resolution: NLT 5 between lindane and *n*-docosane

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lindane ($\gamma\text{-C}_6\text{H}_6\text{Cl}_6$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of lindane to *n*-docosane in the *Sample solution*

R_S = peak response ratio of lindane to *n*-docosane in the *Standard solution*

C_S = concentration of USP Lindane RS in the *Standard stock solution* (mg/mL)

C_U = nominal concentration of lindane in the *Sample stock solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• PH (791)

Sample solution: 1-in-5 dilution

Acceptance criteria: 8.0–9.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**
USP Lindane RS

Lindane Lotion

» Lindane Lotion is Lindane in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lindane ($\gamma\text{-C}_6\text{H}_6\text{Cl}_6$).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Lindane RS

Identification—It responds to the *Identification* test under *Lindane Cream*.

pH (791): between 6.5 and 8.5.

Assay—Proceed as directed in the *Assay* under *Lindane Cream*, substituting "Lotion" for "Cream" throughout.

Lindane Shampoo

DEFINITION

Lindane Shampoo is Lindane in a suitable vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of lindane ($\gamma\text{-C}_6\text{H}_6\text{Cl}_6$).

IDENTIFICATION

• A.

Analysis: Wind a 1.5-cm × 5-cm strip of 20-mesh copper gauze around the end of a copper wire. Heat the

gauze in the nonluminous flame of a Bunsen burner until it glows, without coloring the flame green. Allow the gauze to cool, and repeat the heating and cooling step several times until a thorough coating of oxide is formed. Apply a small amount of Shampoo to the cooled gauze, ignite, and allow to burn freely in the air. Hold the gauze in the outer edge of the burner flame at a height of 4 cm.

Acceptance criteria: A bright green color is imparted to the flame.

ASSAY

PROCEDURE

Mobile phase: Anhydrous ethyl ether and chromatographic solvent hexane (18:280)

Internal standard solution: 1 mg/mL of *n*-docosane in methylene chloride

Standard stock solution: 2 mg/mL of USP Lindane RS in methylene chloride

Standard solution: Transfer 5.0 mL of *Standard stock solution* to a graduated centrifuge tube, add 5.0 mL of *Internal standard solution*, and evaporate with the aid of gentle heat and a current of dry air to 3 mL. Avoid evaporating to dryness. If the mixture is inadvertently evaporated to dryness, discard it, and begin another *Standard solution*.

Solid support: Use 60- to 100-mesh magnesium silicate that has been previously heated at 300° for 2 h.

Sample stock solution: Nominally 2 mg/mL of lindane from a quantity of Shampoo, equivalent to 10 mg of lindane, prepared as follows. Place a pledget of cotton on a removable porous plate at the base of a 25-mm × 200-mm chromatographic column that is fitted with a polytetrafluoroethylene stopcock. Add 50 mL of *Mobile phase* and 10 g of *Solid support*, and stir the mixture to expel air bubbles. Add 1.5 g of anhydrous sodium sulfate to the column, and elute until the surface of the liquid is 4 cm above *Solid support*, discarding the eluate. Transfer a weighed portion of Shampoo, corresponding to 10 mg of lindane, to a 150-mL beaker, and add 10 g of *Solid support*. Mix with a spatula, adding chromatographic solvent hexane as necessary to produce a homogeneous mixture, and continue stirring until a free-flowing powder is produced. Transfer this mixture to the chromatographic column with the aid of three 5-mL portions of *Mobile phase*. Elute the column with 225 mL of *Mobile phase* at a flow rate of 2–3 mL/min, collecting the eluate in a 250-mL beaker. Remove the chromatographic column, add 5.0 mL of *Internal standard solution* to the eluate, and evaporate with the aid of gentle heat and a current of dry air to 5 mL.

Sample solution: Transfer the *Sample stock solution* to a graduated centrifuge tube with the aid of 1 mL of methylene chloride, and evaporate with the aid of gentle heat and a current of dry air to 3 mL. Avoid evaporating to dryness. If the mixture is inadvertently evaporated to dryness, discard it, and begin another *Sample solution*.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 1.8-m × 2-mm glass; packed with 3% liquid phase G3 on support S1A

Temperatures

Injector: 250°

Detector: 250°

Column: 195°

Carrier gas: Dry nitrogen

Flow rate: 40 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 5 between lindane and *n*-docosane

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0% for 6–10 replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lindane (γ -C₆H₆Cl₆) in the portion of Shampoo taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of lindane to *n*-docosane from the *Sample solution*

R_S = peak response ratio of lindane to *n*-docosane from the *Standard solution*

C_S = concentration of USP Lindane RS in the *Standard stock solution* (mg/mL)

C_U = nominal concentration of lindane in the *Sample stock solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

pH (791): 6.2–7.0

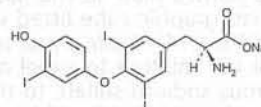
ADDITIONAL REQUIREMENTS

PACKAGING AND STORAGE: Preserve in tight containers.

USP REFERENCE STANDARDS (11)

USP Lindane RS

Liothyronine Sodium



C₁₅H₁₁I₃NNaO₄ 672.96

L-Tyrosine, O-(4-hydroxy-3-iodophenyl)-3,5-diiodo-, monosodium salt.

Monosodium L-3-[4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl]alanine [55-06-1].

» Liothyronine Sodium is the sodium salt of L-3,3',5-triiodothyronine. It contains not less than 95.0 percent and not more than 101.0 percent of C₁₅H₁₁I₃NNaO₄, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Levothyroxine RS

USP Liothyronine RS

Identification—

A: The UV absorption spectrum of a 1 in 10,000 solution in dilute hydrochloric acid (1 in 50) in 80 percent alcohol exhibits maxima at the same wavelengths as that of a similar solution of USP Liothyronine RS, concomitantly measured; and the respective absorptivities, both calculated on the dried basis in terms of the acid, at the wavelength of maximum absorbance at about 297 nm, do not differ by more than 5.0%.

B: Heat about 50 mg with a few drops of sulfuric acid in a porcelain crucible: violet vapors of iodine are evolved.

C: The residue from the ignition of it meets the requirements of the tests for *Sodium* (191).

D: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Specific rotation (781S): between +18° and +22°.

Test solution: 20 mg per mL, in a mixture of alcohol and 1.2 N hydrochloric acid (4:1).

Loss on drying (731)—Dry it at 105° for 2 hours: it loses not more than 4.0% of its weight.

Limit of levothyroxine sodium—

Mobile phase and Chromatographic system—Proceed as directed in the *Assay*.

Standard solution—Proceed as directed for *Standard preparation* in the *Assay*.

Test solution—Proceed as directed for *Assay preparation* in the *Assay*.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the levothyroxine peak responses. Calculate the percentage of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) in the portion of Liothyronine Sodium taken by the formula:

$$100(798.85/776.87)(C_S / C_T)(r_U / r_S)$$

in which 798.85 and 776.87 are the molecular weights of levothyroxine sodium and levothyroxine, respectively; C_S is the concentration, in µg per mL, of USP Levothyroxine RS in the *Standard solution*; C_T is the concentration, in µg per mL, of Liothyronine Sodium in the *Assay preparation*; and r_U and r_S are the levothyroxine peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 5.0% of levothyroxine sodium is found.

Chloride content—Weigh accurately 100 mg, previously dried, and transfer to a platinum dish. Ignite over a low flame, protecting the dish from air currents during the ignition. When carbonization is complete, cool the dish, add 2 drops of water, and break up the charred mass thoroughly with a stirring rod. Add 10 mL of water and 5 mL of ammonium hydroxide, and mix. Transfer the slurry to a glass-stoppered, 50-mL flask, and wash the platinum dish and the stirring rod with water, adding the washings to the flask, until the volume of the solution is about 25 mL. Add 10 mL of silver nitrate solution (1 in 20), shake thoroughly, and filter through a retentive paper into a 50-mL color-comparison tube. Wash the flask and the filter paper with 10 mL of water, and add the washings to the tube. Acidify the combined filtrate and washings to litmus with nitric acid, and dilute with water to 50 mL. Prepare a control by mixing 5 mL of ammonium hydroxide, 20 mL of water, and 10 mL of silver nitrate solution (1 in 20), filtering the mixture through a retentive paper into a 50-mL color-comparison tube, then washing the filter paper with 10 mL of water into the tube, acidifying the contents of the tube to litmus with nitric acid, diluting with water to 50 mL, and adding sodium chloride solution (1 in 1000) in 0.1-mL increments until the turbidity of the control matches that of the test solution. Not more than 2.0 mL of sodium chloride is required (1.2%).

Sodium content—Weigh accurately about 100 mg, previously dried, and transfer to a platinum dish. Add 8 to 10 drops of sulfuric acid, and ignite to constant weight, taking care to avoid spattering. Each mg of residue is equivalent to 0.324 mg of Na. Correct the result for the amount of sodium equivalent to the NaCl found in the test for *Chloride content*: not less than 2.9% and not more than 4.0% is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (60:40) that contains 0.5 mL of phosphoric acid in each 1000 mL. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

0.01 M Methanolic sodium hydroxide—Dissolve 400 mg of sodium hydroxide in 500 mL of water. Cool, add 500 mL of methanol, and mix.

Liothyronine stock solution—Dissolve an accurately weighed quantity of USP Liothyronine RS in *0.01 M Methanolic sodium hydroxide* to obtain a solution having a known concentration of about 0.4 mg of liothyronine per mL.

Levothyroxine stock solution—Dissolve an accurately weighed quantity of USP Levothyroxine RS in *0.01 M Methanolic sodium hydroxide* to obtain a solution having a known concentration of about 0.4 mg of levothyroxine per mL. Make a 1:100 dilution of this solution using *Mobile phase*.

Standard preparation—Transfer appropriate volumes of *Liothyronine stock solution* and *Levothyroxine stock solution* to a suitable container, and dilute quantitatively and stepwise, if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 10 µg of liothyronine per mL and 0.5 µg of levothyroxine per mL.

Assay preparation—Prepare a solution of Liothyronine Sodium in *Mobile phase* having a known concentration of about 10 µg per mL. [NOTE—A small amount of *0.01 M Methanolic sodium hydroxide* can be used to facilitate the dissolution of the sample.]

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R , between levothyroxine and liothyronine is not less than 5.0; and the relative standard deviation for replicate injections is not more than 2.0% for liothyronine.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{15}H_{11}I_3NNaO_4$ in the portion of Liothyronine Sodium taken by the formula:

$$100(672.96/650.97)(C_S / C_T)(r_U / r_S)$$

in which 672.96 and 650.97 are the molecular weights of liothyronine sodium and liothyronine, respectively; C_S is the concentration, in µg per mL, of USP Liothyronine RS in the *Standard preparation*; C_T is the concentration, in µg per mL, of Liothyronine Sodium in the *Assay preparation*; and r_U and r_S are the liothyronine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Liothyronine Sodium Tablets

» Liothyronine Sodium Tablets contain an amount of $C_{15}H_{11}I_3NNaO_4$ equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of liothyronine ($C_{15}H_{12}I_3NO_4$).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Levothyroxine RS

USP Liothyronine RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to

the liothyronine peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)—[NOTE—All containers that are in contact with solutions containing liothyronine sodium are to be made of glass.]

Medium: pH 10.0 ± 0.05 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 250 mL.

Apparatus 3: 30 dips per minute, using 20-mesh screen on the top and 40-mesh screen on the bottom of the glass reciprocating cylinder.

Time: 45 minutes.

Determine the amount of liothyronine sodium ($C_{15}H_{12}I_3NO_4$) dissolved by employing the following method.

Ammoniated solution—Add 0.05 mL of ammonium hydroxide to 200 mL of water.

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (55:45) that contains 1 mL of phosphoric acid in each 1000 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard solution—Dissolve an accurately weighed quantity of USP Liothyronine RS in *Ammoniated solution*, and dilute quantitatively, and stepwise if necessary, with *Ammoniated solution* to obtain a solution having a known concentration of about 10 µg of USP Liothyronine RS per mL. Dilute a portion of this solution quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.5 µg of USP Liothyronine RS per mL.

Test solution—Transfer 20 mL of the solution under test to a centrifuge tube, and centrifuge until a clear supernatant is obtained.

Resolution solution—Prepare a solution of USP Liothyronine RS and USP Levothyroxine RS in *Ammoniated solution* having known concentrations of about 10 µg of each USP Reference Standard per mL. Dilute with water to obtain a concentration of about 0.5 µg of each USP Reference Standard per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between liothyronine and levothyroxine is not less than 3.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 4.0%.

Procedure—Separately inject equal volumes (about 200 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of $C_{15}H_{12}I_3NO_4$ dissolved.

Tolerances—Not less than 70% (Q) of the labeled amount of $C_{15}H_{12}I_3NO_4$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay* under *Liothyronine Sodium*.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 µg of liothyronine sodium, to a centrifuge tube, add 2 glass beads, pipet 10 mL of *Mobile phase* into the tube, and mix using a vortex mixer for 3 minutes. Centrifuge to obtain a clear supernatant, filtering if necessary.

Procedure—Proceed as directed in the *Assay* under *Liothyronine Sodium*. Calculate the quantity, in µg, of

liothyronine ($C_{15}H_{12}I_3NO_4$) in the portion of Tablets taken by the formula:

$$10C(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Liothyronine RS in the *Standard preparation*; and *r_U* and *r_S* are the liothyronine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Liotrix Tablets

» Liotrix Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of Levothyroxine Sodium ($C_{15}H_{10}I_4NNaO_4$) and Liothyronine Sodium ($C_{15}H_{11}I_3NNaO_4$) in a ratio by weight of 4 to 1, respectively.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Levothyroxine RS

USP Liothyronine RS

Identification—The retention time of the two major peaks in the chromatogram of the *Assay preparation* corresponds to the levothyroxine and liothyronine peaks in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Disintegration (701): 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (65:35) that contains 2 mL of trifluoroacetic acid in each 1000 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

0.01 M Methanolic sodium hydroxide—Dissolve 400 mg of sodium hydroxide in 500 mL of water. Cool, add 500 mL of methanol, and mix.

Levothyroxine stock solution—Dissolve an accurately weighed quantity of USP Levothyroxine RS in *0.01 M Methanolic sodium hydroxide* to obtain a solution having a known concentration of about 0.4 mg of levothyroxine per mL.

Liothyronine stock solution—Dissolve an accurately weighed quantity of USP Liothyronine RS in *0.01 M Methanolic sodium hydroxide* to obtain a solution having a known concentration of about 0.4 mg of liothyronine per mL. Make a 1:10 dilution of this solution using *Mobile phase*.

Standard preparation—Transfer appropriate volumes of *Levothyroxine stock solution* and *Liothyronine stock solution* to a suitable container, and dilute quantitatively and stepwise, if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 10 µg of levothyroxine per mL and 2.5 µg of liothyronine per mL.

Assay preparation—Transfer 20 Tablets to a 200-mL volumetric flask, add 180 mL of *Mobile phase*, and sonicate for 15 minutes, occasionally swirling the flask to accelerate the disintegration of the Tablets. Cool to room temperature, and dilute with *Mobile phase* to volume. Transfer a portion of the solution to a centrifuge tube, and centrifuge for 10 minutes at 5000 rpm. Quantitatively dilute a portion of the clear supernatant with *Mobile phase* to obtain concentrations of about 10.0 µg of levothyroxine sodium per mL and 2.5 µg of liothyronine sodium per mL.

Chromatographic system—Proceed as directed in the *Assay* under *Levothyroxine Sodium*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Levothyroxine Sodium*. Calculate the quantity, in μg , of levothyroxine sodium ($\text{C}_{15}\text{H}_{10}\text{I}_4\text{NNaO}_4$) in the portion of Tablets taken by the formula:

$$(798.86/776.87)(10C)(r_U / r_S)$$

in which 798.86 and 776.87 are the molecular weights of levothyroxine sodium and levothyroxine, respectively; C is the concentration, in μg per mL, of USP Levothyroxine RS in the *Standard preparation*; and r_U and r_S are the levothyroxine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Calculate the quantity, in μg of liothyronine sodium ($\text{C}_{15}\text{H}_{11}\text{I}_3\text{NNaO}_4$) in the portion of Tablets taken by the formula:

$$(672.96/650.98)(10C)(r_U / r_S)$$

in which 672.96 and 650.98 are the molecular weights of liothyronine sodium and liothyronine, respectively; C is the concentration, in μg per mL, of USP Liothyronine RS in the *Standard preparation*; and r_U and r_S are the liothyronine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Lipid Injectable Emulsion

» Lipid Injectable Emulsion used in total parenteral nutrition is a sterile 10 (0.10 g per mL), 20 (0.20 g per mL), or 30 (0.30 g per mL) percent w/v emulsion in an aqueous vehicle. The aqueous phase contains 0.6 percent to 1.8 percent w/v parenteral Egg Phospholipids in Water for Injection and contains, if necessary, an osmotic agent, such as glycerin in amounts of 1.7 percent to 2.5 percent w/v, or a suitable stabilizer, such as a fatty acid salt. The most frequently used oil present is Soybean Oil, which provides an ample supply of the essential fatty acids: linoleic acid and linolenic acid. Other oils, such as Safflower Oil, Medium-Chain Triglycerides, Olive Oil, Fish Oil, or other suitable oils, can be mixed with Soybean Oil. Hence, Soybean Oil can be the only oil or be part of a mixture of these other oils. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of the total oil(s). It contains no antimicrobial agents. The final products are terminally sterilized.

Packaging and storage—Preserve in an appropriate container (see *Packaging and Storage Requirements* (659), *Injection Packaging*). Use elastomeric closures that are compatible with both the oil and water phases of the Emulsion. Store at a temperature not below 4° (protect from freezing) or above 30° (protect from excessive heat).

Labeling—The label states the identity and the quantities of the specific oils in the Emulsion. The label states the total osmolar concentration (or osmolarity) in mOsm per L. The labeling provides the following information: do not use if there is evidence of excessive creaming or aggregation, if excessive free oil droplets are visible, or if there are other indications of compromised integrity, such as microbial growth, present in the product.

USP Reference standards (11)—
USP Endotoxin RS

Fatty acid composition—Transfer a volume of the Emulsion, equivalent to about 200 mg of lipids, to a stoppered extraction vessel, add 10 mL of ether, and mix. Add 5 g of anhydrous sodium sulfate, mix, and allow the mixture to stand until separation of the layers is complete. Wet the packing of a chromatographic silica cartridge with a few mL of ether, transfer about 5 mL of the ether layer from the extraction vessel to the column reservoir, and elute at a rate of between 5 and 10 drops per minute into a suitable vessel. Evaporate the ether from the eluant, and dissolve the residue in 5.0 mL of toluene. Transfer 1.0 mL of the toluene solution to a reaction vial, and add 0.4 mL of (*m*-trifluoromethylphenyl) trimethylammonium hydroxide in methanol. Cover, mix, and allow to stand for 30 minutes. Inject about 1 μL of this solution into a gas chromatograph equipped with a 0.53-mm \times 50-m wide-bore, fused-silica capillary column coated with a 2.0- μm thickness of liquid phase G16 and maintained at a temperature of 200°. The column is connected to a flame-ionization detector. Helium is used as the carrier gas at a flow rate of about 10 mL per minute. Measure the main peak areas of the methyl esters of the fatty acids. The relative peak areas expressed as a percentage of the main peaks are in the known ranges for the oil (e.g., Soybean Oil, USP; Safflower Oil, USP) as specified on the label. For oil mixtures, analysis of each oil should be performed to identify known peaks prior to emulsification as specified on the label.

Bacterial Endotoxins Test (85)—It contains not more than 0.5 USP Endotoxin Unit per mL.

pH (791): between 6.0 and 9.0.

Globule size limits—The Injectable Emulsion meets the requirements of the limits specified in both *Method I* and *Method II* as directed under *Globule Size Distribution in Lipid Injectable Emulsions* (729).

Limit of oil droplet mean diameters (See *Method I—Light Scattering Method* under *Globule Size Distribution in Lipid Injectable Emulsions* (729))—Using the method of light scattering, determine the mean droplet diameter (MDD): the sample meets the requirements. The intensity-weighted mean droplet diameter (MDD) for the Injectable Emulsion must be ≤ 500 nm, or 0.5 μm , irrespective of the concentration of the dispersed lipid phase.

Limit of large globule volume-diameter (See *Method II—Light Obscuration or Extinction Method* under *Globule Size Distribution in Lipid Injectable Emulsions* (729))—Using the method of light obscuration, determine the size distribution of globules in the large-diameter tail of the dispersion (detection threshold ≥ 2.0 μm). Calculate the volume-weighted mass of lipid in the form of globules with diameters in excess of 5.0 μm per 100 mL of the Injectable Emulsion. The volume-weighted, large-diameter fat globule limits of the dispersed phase, expressed as the percentage of fat residing in globules larger than 5 μm (PFAT5) for a given Injectable Emulsion, is not to exceed 0.05%.

Limit of free fatty acid—

Solvent—Prepare a mixture of heptane, isopropanol, and water (400:400:200) in a separatory funnel. Allow the phases to separate, and discard the lower phase. Filter the upper phase (heptane solution) through 40 g of anhydrous sodium sulfate. Store in a tightly capped glass container, and use within 1 week.

Chromatographic column—Prepare a slurry of heptane and chromatographic silica gel having an average pore size of 6 nm, and activate at a temperature of 110° for not less than 1 hour prior to use. Transfer the slurry to a 2.3-cm chromatographic tube (see *Column Chromatography* under *Chromatography* (621)), and pack to a bed height of between 5 cm and 6 cm. Wash the column with about 40 mL of heptane, and drain the heptane through the column to a level of about 0.5 cm above the silica gel bed.

Procedure—Transfer 20.0 mL of the Injectable Emulsion to a flask, freeze, and lyophilize. Dissolve the residue in 30 mL of *Solvent*, and transfer the solution to the column. Rinse

the flask with three 30-mL portions of *Solvent*, and transfer the washings to the column, allowing each rinsing to drain to the top of the column bed before applying the next rinse. Collect a total of 120 mL of effluent. Add 10 drops of phenolphthalein TS to the effluent, bubble nitrogen through the solution, and titrate with 0.02 N alcoholic potassium hydroxide VS until the solution remains pale pink after mixing for 10 seconds. Titrate a blank using 120 mL of *Solvent*. Calculate the quantity, in mEq, of free fatty acids per g of oil in the Injectable Emulsion using the formula:

$$(V_U - V_B)N / 20C$$

in which V_U is the volume, in mL, of 0.02 N alcoholic potassium hydroxide consumed by the eluant; V_B is the volume, in mL, of 0.02 N alcoholic potassium hydroxide consumed by the blank; N is the normality of the 0.02 N alcoholic potassium hydroxide; and C is the labeled concentration, in g per mL, of the total oil(s) in the Injectable Emulsion: not more than 0.07 mEq of free fatty acids per g of oil is found.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Mobile phase—Prepare a filtered and degassed mixture of isopropanol, ethyl acetate, and glacial acetic acid (179:20:1).

Standard preparation—Dissolve an accurately weighed portion of Soybean Oil (or other relevant oils used in the Emulsion) in *Mobile phase* to obtain a solution having a known concentration of about 8 mg per mL.

Assay preparation—Transfer an accurately measured portion of Emulsion, equivalent to about 800 mg of oil, to a 100-mL volumetric flask with the aid of additional portions of *Mobile phase*. Dilute with *Mobile phase* to volume, and mix to obtain a solution containing about 8 mg of oil per mL.

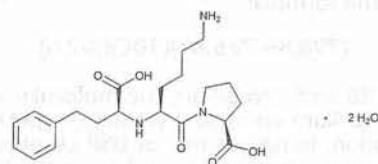
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a refractive index detector and a 4.1-mm × 25-cm column that contains packing L21. The flow rate is about 1 mL per minute, adjusted so that the peak due to oil elutes at about 6.5 minutes. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor, k' , is not less than 1.0; the tailing factor for the oil peak is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of oil in the portion of Emulsion taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of Soybean Oil or other relevant oils used in the Emulsion in the *Standard preparation*; and r_U and r_S are the Emulsion responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Lisinopril



$C_{21}H_{31}N_3O_5 \cdot 2H_2O$ 441.52
L-Proline, 1-[*N*²-(1-carboxy-3-phenylpropyl)-L-lysyl]-, dihydrate, (*S*)-;
1-[*N*²-[(*S*)-1-Carboxy-3-phenylpropyl]-L-lysyl]-L-proline dihydrate [83915-83-7].

DEFINITION

Lisinopril contains NLT 98.0% and NMT 102.0% of lisinopril ($C_{21}H_{31}N_3O_5$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: 2.76 g/L of monobasic sodium phosphate in water prepared as follows. Dissolve 2.76 g of monobasic sodium phosphate in about 900 mL of water in a 1000-mL volumetric flask. Adjust with 1 N sodium hydroxide to a pH of 5.0 and dilute with water to volume.

Mobile phase: Acetonitrile and *Solution A* (4:96)

Standard solution: 0.3 mg/mL of USP Lisinopril RS in water

Sample solution: 0.3 mg/mL of Lisinopril in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L7

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.7

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lisinopril ($C_{21}H_{31}N_3O_5$) in the portion of Lisinopril taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

r_U = peak response of lisinopril from the *Sample solution*

r_S = peak response of lisinopril from the *Standard solution*

C_S = concentration of USP Lisinopril RS in the *Standard solution* (mg/mL)

C_U = concentration of Lisinopril in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS**, *Method II* (231): 0.001% (Official 1-Jan-2018)
- **ORGANIC IMPURITIES**
 Buffer: 3.53 g/L of monobasic sodium phosphate dihydrate in water adjusted with phosphoric acid to a pH of 4.1
 Solution A: Acetonitrile and Buffer (7:193)
 Solution B: Acetonitrile and Buffer (20:80)
 Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	60	40
55	60	40
60	100	0

Standard solution: 0.006 mg/mL of USP Lisinopril RS in Solution A

Sensitivity solution: 1.0 µg/mL of USP Lisinopril RS in Solution A from Standard solution

Sample solution: 2 mg/mL of Lisinopril in Solution A

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 45°

Flow rate: 1.8 mL/min

Injection volume: 20 µL

System suitability

Samples: Standard solution and Sensitivity solution

Suitability requirements

Relative standard deviation: NMT 10.0%, Standard solution

Signal-to-noise ratio: NLT 10, Sensitivity solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Lisinopril taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each impurity from the Sample solution

r_s = peak response of lisinopril from the Standard solution

C_s = concentration of USP Lisinopril RS in the Standard solution (mg/mL)

C_u = concentration of Lisinopril in the Sample solution (mg/mL)

F = relative response factor (see Table 2)

Acceptance criteria: See Table 2. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-Alkyl-L-lysine ^a	0.57	0.35	0.3
DL-Homophenylalanine ^b	0.72	1.08	0.30
Lisinopril	1.00	1.00	—
Lisinopril epimer ^c	1.33	0.76	0.3
Lisinopril cyclohexyl analog ^d	2.93	0.39	0.30
R,S,S-Diketopiperazine ^e	3.88	0.79	0.3
S,S,S-Diketopiperazine (lisinopril related compound A) ^f	4.04	0.76	0.3
N-Alkyl lisinopril ^g	4.60	0.86	0.15
Any individual unspecified impurity	—	1.00	0.1
Total impurities ^h	—	—	0.5

^a [(S)-1-Carboxy-3-phenylpropyl]-L-lysine.

^b 2-Amino-4-phenylbutanoic acid.

^c [(R)-1-Carboxy-3-phenylpropyl]-L-lysyl-L-proline.

^d [(S)-1-Carboxy-3-cyclohexylpropyl]-L-lysyl-L-proline.

^e (S)-2-[(3S,8aR)-3-(4-Aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-d]pyrazin-2(1H)-yl]-4-phenylbutanoic acid.

^f (S)-2-[(3S,8aS)-3-(4-Aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-d]pyrazin-2(1H)-yl]-4-phenylbutanoic acid.

^g N^ε,N^ε-Bis[(S)-1-Carboxy-3-phenylpropyl]-L-lysyl-L-proline.

^h Total impurities does not include lisinopril epimer.

SPECIFIC TESTS

- **OPTICAL ROTATION** (781S), *Specific Rotation*

Diluent: 0.25 M zinc acetate solution prepared as follows. Mix 600 mL of water with 150 mL of glacial acetic acid and 54.9 g of zinc acetate, and stir to dissolve the zinc acetate. While stirring, add 150 mL of ammonium hydroxide, cool to room temperature, and adjust with ammonium hydroxide to a pH of 6.4. Transfer the solution to a 1000-mL volumetric flask, and dilute with water to volume.

Sample solution: 10 mg/mL of Lisinopril in Diluent

Acceptance criteria: −115.3° to −122.5° (λ = 405 nm)

- **WATER DETERMINATION** (921), *Method I*: 8.0%–9.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)
USP Lisinopril RS

Lisinopril Compounded Oral Suspension**DEFINITION**

Lisinopril Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of lisinopril (C₂₁H₃₁N₃O₅).

Prepare Lisinopril Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Lisinopril tablets ^a equivalent to	100 mg of lisinopril
Vehicle: a 1:1 mixture of Ora-Sweet ^b and Ora-Plus, ^b a sufficient quantity to make	100 mL

^a Prinivil 10-mg tablets, Merck & Co., West Point, PA.

^b Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Lisinopril tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a lisinopril liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

ASSAY**• PROCEDURE**

Solution A: 4.1 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.0.

Mobile phase: 1.0 g/L of sodium 1-hexanesulfonate in acetonitrile and *Solution A* (18:82). Filter and degas.

Diluent: Methanol and water (20:80)

Standard solution: 0.2 mg/mL of USP Lisinopril RS in *Diluent*

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Mix 1.0 mL of Oral Suspension with 4.0 mL of *Diluent* to obtain a solution having a nominal concentration of 0.2 mg/mL of lisinopril.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for lisinopril is about 12.9 min.]

Suitability requirements

Column efficiency: NLT 1800 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lisinopril RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lisinopril in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- pH (791):** 4.3–5.3

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
- BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator or at controlled room temperature
- LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.

• USP REFERENCE STANDARDS (11)

USP Lisinopril RS

Lisinopril Tablets

» Lisinopril Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{31}N_3O_5$.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Lisinopril RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of lisinopril dissolved using the following method.

Mobile phase and Chromatographic system—Prepare as directed in the *Assay*.

Determine the amount of lisinopril dissolved by one of the following procedures.

PROCEDURE FOR POOLED SAMPLE—Proceed as directed for *Procedure* in *Apparatus 1* and *Apparatus 2*, *Immediate-Release Dosage Forms* under *Dissolution* (711). Combine equal volumes of the filtered solutions of the 6 or 12 individual specimens withdrawn, and use the pooled sample as the test solution. Inject a volume of the pooled sample into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the quantity of $C_{21}H_{31}N_3O_5$ dissolved in comparison with a *Standard solution* having a known concentration of USP Lisinopril RS in the same *Medium* and similarly chromatographed.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{21}H_{31}N_3O_5$ in the Tablets is dissolved in 30 minutes: the requirements are met if the quantities of active ingredient dissolved from the pooled sample conform to the accompanying *Acceptance Table for a Pooled Sample*. Continue testing through the three stages unless the results conform at either S_1 or S_2 . The quantity, Q, is the amount of dissolved active ingredient specified, expressed as a percentage of the labeled content.

Acceptance Table for a Pooled Sample

Stage	Number Tested	Acceptance Criteria
S_1	6	Average amount dissolved is not less than Q + 10%.
S_2	6	Average amount dissolved ($S_1 + S_2$) is equal to or greater than Q + 5%.
S_3	12	Average amount dissolved ($S_1 + S_2 + S_3$) is equal to or greater than Q.

PROCEDURE FOR UNIT SAMPLE—Proceed as directed for *Procedure* in *Apparatus 1* and *Apparatus 2*, *Immediate-Release Dosage Forms* under *Dissolution* (711). Inject a volume of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the amount of $C_{21}H_{31}N_3O_5$ dissolved in comparison with a *Standard solution* having a known concentration of USP Lisinopril RS in the *Medium* and similarly chromatographed.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{21}H_{31}N_3O_5$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Procedure for content uniformity—

Phosphate solution, Mobile phase, and Chromatographic system—Prepare as directed in the Assay.

Diluent—Dissolve 2.72 g of monobasic potassium phosphate in 800 mL of water, adjust with phosphoric acid to a pH of 4.0, dilute with water to 1000 mL, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Lisinopril RS in *Diluent* to obtain a solution having a known concentration of about 0.2 mg per mL.

Test preparation—Place one Tablet in a volumetric flask of appropriate size, based on the labeled quantity, in mg, of lisinopril in the Tablet, to obtain a solution containing 0.2 mg of lisinopril per mL. Fill the flask to about 50% volume with *Diluent*, sonicate for 5 minutes, and shake by mechanical means for 20 minutes. Dilute with *Diluent* to volume, mix, and filter.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 μ L) of the *Test preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{31}N_3O_5$ in the Tablet taken by the formula:

$$(TC / D)(r_U / r_S)$$

in which *T* is the labeled quantity, in mg, of lisinopril in the Tablet; *C* is the concentration, in mg per mL, calculated on the anhydrous basis, of USP Lisinopril RS in the *Standard preparation*; *D* is the concentration, in mg per mL, of lisinopril in the *Test preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and *r_U* and *r_S* are the lisinopril peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively.

Related compounds—

Phosphate solution, Mobile phase, Diluent, and Chromatographic system—Prepare as directed in the Assay.

Standard solution—Dilute the *Standard preparation*, prepared as directed in the Assay, with *Diluent* to obtain a solution having a known concentration of about 20 μ g per mL.

Test solution—Use the Assay preparation.

Procedure—Proceed as directed in the Assay. Measure the responses of the lisinopril peak obtained from the *Standard solution*, and of all peaks other than that of lisinopril obtained from the *Test solution*. Calculate the percentage of related compounds in each Tablet taken by the formula:

$$100(V / 10)(C / L)(r_U / r_S)$$

in which *V* is the volume, in mL, of the *Test solution*; *C* is the concentration, in mg per mL, calculated on the anhydrous basis, of USP Lisinopril RS in the *Standard solution*; *L* is the quantity, in mg, of lisinopril in each Tablet, taken as determined in the Assay; *r_U* is the sum of all peak responses other than that of lisinopril obtained from the *Test solution*; and *r_S* is the lisinopril peak response obtained from the *Standard solution*: not more than 2.0%, calculated on the basis of the quantity, in mg, of lisinopril in the portion of Tablets taken, as determined in the Assay, is found.

Assay—

Phosphate solution—Dissolve 4.1 g of monobasic potassium phosphate in about 900 mL of water in a 1000-mL volumetric flask, and adjust with phosphoric acid to a pH of 2.0. Dilute with water to volume, and mix.

Mobile phase—Dissolve 1.0 g of sodium 1-hexanesulfonate in 820 mL of *Phosphate solution*. Add 180 mL of acetonitrile, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Diluent—Prepare a mixture of water and methanol (4:1).

Standard preparation—Dissolve an accurately weighed quantity of USP Lisinopril RS in *Diluent* to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer to a suitable size volumetric flask 10 Tablets, which when diluted with *Diluent* will yield a solution having a concentration of about 0.2 mg per mL. Add *Diluent*, and sonicate for 5 minutes. Shake the flask by mechanical means for 20 minutes, dilute with *Diluent* to volume, mix, and filter.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm \times 20-cm column that contains packing L7 and is maintained at a temperature of 40°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 700 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; the capacity factor, *k'*, for the analyte peak is greater than 1.5; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{31}N_3O_5$ in each Tablet taken by the formula:

$$(L/D)C(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of lisinopril in each Tablet, *D* is the concentration, in mg per mL, of lisinopril in the *Assay preparation* based on the labeled quantity per Tablet and the extent of dilution; *C* is the concentration, in mg per mL, calculated on the anhydrous basis, of USP Lisinopril RS in the *Standard preparation*; and *r_U* and *r_S* are the lisinopril peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Lisinopril and Hydrochlorothiazide Tablets

DEFINITION

Lisinopril and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

- A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

The Tablets must be assayed within 24 h of the time they are put in the solution.

Buffer: Dissolve 4.08 g of monobasic potassium phosphate in 800 mL of water. Adjust with phosphoric acid to a pH of 2.5, and dilute with water to 1 L.

Mobile phase: Acetonitrile, triethylamine, water, and phosphoric acid (280:3:1480:15)

Lisinopril standard stock solution: 0.5 mg/mL of USP Lisinopril RS in *Buffer*

Hydrochlorothiazide standard stock solution: 3.12 mg/mL of USP Hydrochlorothiazide RS in methanol

Lisinopril related compound A stock solution: 0.05 mg/mL of USP Lisinopril Related Compound A RS in methanol

Benzothiadiazine related compound A stock solution: 0.016 mg/mL of USP Benzothiadiazine Related Compound A RS in methanol

Standard solution: 0.1 mg/mL of USP Lisinopril RS, 0.125 mg/mL of USP Hydrochlorothiazide RS, 2 µg/mL of USP Lisinopril Related Compound A RS, and 1.3 µg/mL of USP Benzothiadiazine Related Compound A RS in Buffer from Lisinopril standard stock solution, Hydrochlorothiazide standard stock solution, Lisinopril related compound A stock solution, and Benzothiadiazine related compound A stock solution. For Tablet strengths 20/12.5 of lisinopril/hydrochlorothiazide, the concentration of USP Lisinopril RS and USP Lisinopril Related Compound A RS in the Standard solution is 0.2 mg/mL and 4 µg/mL, respectively.

Sample stock solution: Transfer 10 Tablets to a suitable volumetric flask. Add Buffer (0.25 mL/mg of total lisinopril), sonicate for 5 min, and then add methanol (0.5 mL/mg of total lisinopril). Sonicate for an additional 10 min. Add more Buffer (0.75 mL/mg of total lisinopril), and mix by mechanical means for 20 min. Dilute with water to volume to prepare solutions as described in Table 1.

Table 1

Tablet Strength of Lisinopril/Hydrochlorothiazide (mg/Tablet)	Nominal Concentration of Lisinopril/Hydrochlorothiazide (mg/mL)
10/12.5	0.4/0.5
20/12.5	0.4/0.25
20/25	0.4/0.5

Sample solution: Dilute the Sample stock solution with Buffer to prepare solutions as described in Table 2. Pass a portion through a suitable filter of 0.45-µm pore size.

Table 2

Tablet Strength of Lisinopril/Hydrochlorothiazide (mg/Tablet)	Nominal Concentration of Lisinopril/Hydrochlorothiazide (mg/mL)
10/12.5	0.1/0.12
20/12.5	0.2/0.125
20/25	0.1/0.12

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: NLT 10 min

System suitability

Sample: Standard solution

Suitability requirements

Resolution: NLT 3.0 between lisinopril and benzothiadiazine related compound A and NLT 4.0 between hydrochlorothiazide and benzothiadiazine related compound A

Tailing factor: NMT 2 for both the lisinopril and hydrochlorothiazide peaks

Relative standard deviation: NMT 2.0% for both the lisinopril and hydrochlorothiazide peaks

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lisinopril or hydrochlorothiazide from the Sample solution

r_S = peak response of lisinopril or hydrochlorothiazide from the Standard solution

C_S = concentration of USP Lisinopril RS or USP Hydrochlorothiazide RS in the Standard solution (mg/mL)

C_U = nominal concentration of lisinopril or hydrochlorothiazide in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 45 min for hydrochlorothiazide; 30 min for lisinopril

Buffer and Mobile phase: Prepare as directed in the Assay.

Lisinopril standard stock solution: 0.5 mg/mL of USP Lisinopril RS in Buffer

Hydrochlorothiazide standard stock solution: 0.44 mg/mL of USP Hydrochlorothiazide RS in methanol

Standard solution: Prepare solutions in Medium as described in Table 3 from the Lisinopril standard stock solution and Hydrochlorothiazide standard stock solution.

Table 3

Tablet Strength of Lisinopril/Hydrochlorothiazide (mg/Tablet)	Nominal Concentration of Lisinopril/Hydrochlorothiazide (mg/mL)
10/12.5	0.01/0.013
20/12.5	0.02/0.013
20/25	0.02/0.026

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, and discard the first few mL of the filtrate.

Chromatographic system: Proceed as directed in the Assay, except use an injection volume of 20 µL.

System suitability

Sample: Standard solution

Suitability requirements

Resolution: NLT 4.0 between the lisinopril and hydrochlorothiazide peaks

Tailing factor: NMT 2 for both the lisinopril and hydrochlorothiazide peaks

Column efficiency: NLT 6000 theoretical plates for the hydrochlorothiazide peak

Relative standard deviation: NMT 2.0% for both the lisinopril and hydrochlorothiazide peaks

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amounts of lisinopril ($C_{21}H_{31}N_3O_5$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of lisinopril or hydrochlorothiazide from the Sample solution

r_S = peak response of lisinopril or hydrochlorothiazide from the Standard solution

C_S = concentration of lisinopril and hydrochlorothiazide in the Standard solution from Table 3 (mg/mL)

L = labeled amount of lisinopril and hydrochlorothiazide (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amounts of lisinopril ($C_{21}H_{31}N_3O_5$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution* Test 2.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Times: 30 min for both lisinopril and hydrochlorothiazide

Buffer: 2.76 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (6:94)

Standard stock solution 1 (for Tablets labeled to contain 10 mg/12.5 mg or 20 mg/25 mg of lisinopril/hydrochlorothiazide): 0.11 mg/mL of USP Lisinopril RS and 0.14 mg/mL of USP Hydrochlorothiazide RS, prepared as follows. Add acetonitrile to fill 1.5% of the total volume, sonicate until dissolved, and dilute with *Medium* to volume.

Standard stock solution 2 (for Tablets labeled to contain 20 mg/12.5 mg of lisinopril/hydrochlorothiazide): 0.22 mg/mL of USP Lisinopril RS and 0.14 mg/mL of USP Hydrochlorothiazide RS, prepared as follows. Add acetonitrile to fill 1.5% of the total volume, sonicate until dissolved, and dilute with *Medium* to volume.

Standard solution: Prepare solutions, in *Medium*, of lisinopril and hydrochlorothiazide as described in Table 4 from either *Standard stock solution 1* or *Standard stock solution 2*.

Table 4

Tablet Strength of Lisinopril/Hydrochlorothiazide (mg/Tablet)	Nominal Concentration of Lisinopril/Hydrochlorothiazide (mg/mL)
10/12.5	0.011/0.014
20/12.5	0.022/0.014
20/25	0.022/0.028

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Chromatographic system
(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 20-cm; 5- μ m packing L7

Column temperature: 50°

Flow rate: 1.0 mL/min

Injection volume: 50 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.8 for the lisinopril peak and NMT 1.5 for the hydrochlorothiazide peak

Resolution: NLT 3.5 between the lisinopril and hydrochlorothiazide peaks

Relative standard deviation: NMT 2.0% for both peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of lisinopril ($C_{21}H_{31}N_3O_5$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of lisinopril or hydrochlorothiazide from the *Sample solution*

r_S = peak response of lisinopril or hydrochlorothiazide from the *Standard solution*

C_S = concentration of lisinopril or hydrochlorothiazide in the *Standard solution* (mg/mL)

L = labeled amounts of lisinopril or hydrochlorothiazide (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$) and NLT 75% (Q) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Buffer, Mobile phase, Benzothiadiazine related compound A stock solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard solution 1: Use the *Standard solution*, prepared as directed in the *Assay*.

Standard solution 2: 1.28 μ g/mL of USP Benzothiadiazine Related Compound A RS in *Buffer* from the *Benzothiadiazine related compound A stock solution*

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of lisinopril related compound A and benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of lisinopril related compound A or benzothiadiazine related compound A from the *Sample solution*

r_S = peak response of lisinopril related compound A or benzothiadiazine related compound A from *Standard solution 1* or *Standard solution 2*

C_S = concentration of USP Lisinopril Related Compound A RS in *Standard solution 1* or USP Benzothiadiazine Related Compound A RS in *Standard solution 2* (mg/mL)

C_U = nominal concentration of lisinopril or hydrochlorothiazide in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of lisinopril, 405.5, for calculating the percentage of lisinopril related compound A; or molecular weight of hydrochlorothiazide, 297.74, for calculating the percentage of benzothiadiazine related compound A

M_{r2} = molecular weight of lisinopril related compound A, 387.47; or benzothiadiazine related compound A, 285.73

Acceptance criteria

Individual impurities: NMT 2% of lisinopril related compound A; NMT 1% of benzothiadiazine related compound A

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** (11)

USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
 $C_6H_8ClN_3O_4S_2$ 285.73

USP Hydrochlorothiazide RS
 USP Lisinopril RS
 USP Lisinopril Related Compound A RS
 (S)-2-[(3S,8aS)-3-(4-Aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-4-phenylbutanoic acid.
 $C_{21}H_{29}N_3O_4$ 387.47

Lithium Oral Solution

DEFINITION

Lithium Oral Solution is prepared from Lithium Citrate or Lithium Hydroxide to which an excess of Citric Acid has been added. It contains NLT 90.0% and NMT 110.0% of the labeled amount of lithium (Li).

IDENTIFICATION

- **A.** The emission intensity at 671 nm of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Citrate (191):** Meets the requirements

ASSAY

PROCEDURE

Surfactant solution: 1%–2% solution of nonionic surfactant, such as t-dodecyl mercaptan ethoxylate or polyoxyethylene (20) sorbitan monolaurate, in water
Standard stock solution: 0.3 mg/mL of USP Lithium Carbonate RS prepared as follows. Transfer the required quantity of USP Lithium Carbonate RS to a suitable volumetric flask, and add 20% of the flask volume of water and 0.5% of the flask volume of hydrochloric acid. Shake until dissolved.

Standard solution: 6 µg/mL of USP Lithium Carbonate RS from the *Standard stock solution* prepared as follows. Transfer a suitable volume of the *Standard stock solution* to a suitable volumetric flask. Add 80% of the flask volume of water and 2% of the flask volume of *Surfactant solution*, and dilute with water to volume. Determine the pH of the solution.

Sample stock solution: Nominally 0.06 mg/mL of lithium in water prepared as follows. Transfer a volume of Oral Solution equivalent to NLT 60 mg of lithium to a suitable volumetric flask. Dilute with water to volume.

Sample solution: Nominally 1.2 µg/mL of lithium from the *Sample stock solution* prepared as follows. Transfer a suitable volume of the *Sample stock solution* to a suitable volumetric flask. Add 95% of the flask volume of water, 0.2% of the flask volume of 1 N hydrochloric acid, and 2% of the flask volume of the *Surfactant solution*. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to the same pH (± 0.1 pH unit) as that of the *Standard solution*, and dilute with water to volume.

Blank: *Surfactant solution*

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: About 671 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*
 Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of lithium (Li) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (A_r/M_r) \times F \times 100$$

r_U = photometer reading of the *Sample solution*

r_S = photometer reading of the *Standard solution*

C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of lithium in the *Sample solution* (µg/mL)

A_r = atomic weight of lithium, 6.94

M_r = molecular weight of lithium carbonate, 73.89

F = number of lithium ions in one mole of lithium carbonate, 2

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements for oral solution packaged in single-unit containers

SPECIFIC TESTS

- **pH (791):** 4.0–5.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
 USP Lithium Carbonate RS

Lithium Carbonate

Li_2CO_3 73.89
 Carbonic acid, dilithium salt;
 Dilithium carbonate [554-13-2].

DEFINITION

Lithium Carbonate contains NLT 99.0% of lithium carbonate (Li_2CO_3), calculated on the dried basis.

IDENTIFICATION

- **A.** It effervesces upon the addition of an acid, yielding a colorless gas that, when passed into calcium hydroxide TS, immediately forms a white precipitate.
- **B.** When moistened with hydrochloric acid, it imparts an intense crimson color to a nonluminous flame.

ASSAY

PROCEDURE

Sample solution: Dissolve 0.5 g of Lithium Carbonate in 25.0 mL of 1 N hydrochloric acid VS.

Blank: 25.0 mL of 1 N hydrochloric acid VS

Titrimetric system

(See *Titrimetry* (541).)

Mode: Residual titration

Titrant: 1 N sodium hydroxide VS

Endpoint detection: Visual

Indicator: Methyl orange TS

Analysis

Samples: *Sample solution* and *Blank*

Titrate the excess acid in the *Sample solution* with *Titrant*.

Calculate the percentage of lithium carbonate (Li_2CO_3) in the portion of Lithium Carbonate taken:

$$\text{Result} = (V_B - V_S) \times N \times F \times (1/W) \times 100$$

V_B = *Titrant* volume consumed by the *Blank* (mL)

V_S = *Titrant* volume consumed by the *Sample solution* (mL)

N = normality of *Titrant* (mEq/mL)

F = equivalent weight of Lithium Carbonate, 36.95 mg/mEq

W = weight of Lithium Carbonate in the *Sample solution* (mg)

Acceptance criteria: NLT 99.0% on the dried basis

IMPURITIES

ALUMINUM AND IRON

Sample solution: Dissolve 500 mg of Lithium Carbonate in 10 mL of water by the dropwise addition, with agitation, of hydrochloric acid.

Analysis: Boil the *Sample solution*, then cool it. To 5 mL of the solution add 6 N ammonium hydroxide until the reaction is alkaline.

Acceptance criteria: No turbidity or precipitate is observed.

• **CALCIUM**

Sample solution: Suspend 5.0 g of Lithium Carbonate in 50 mL of water, and add a slight excess of 3 N hydrochloric acid. Boil the clear solution to expel carbon dioxide, add 5 mL of ammonium oxalate TS, render alkaline with 6 N ammonium hydroxide, and allow to stand for 4 h. Pass through a filtering crucible, and wash with warm water until the last washing yields no turbidity with calcium chloride TS. Place the crucible in a beaker, cover the crucible with water, add 3 mL of sulfuric acid, and heat to 70°.

Analysis: Titrate the *Sample solution* with 0.10 N potassium permanganate to a pale pink color that persists for 30 s.

Acceptance criteria: NMT 3.8 mL of 0.10 N potassium permanganate is consumed (0.15%).

• **SODIUM**

Standard stock solution: 500 µg/mL of sodium prepared as follows. Dissolve 1.271 g of sodium chloride, previously dried at 130° to constant weight, in water in a 1000-mL volumetric flask. Dilute with water to volume.

Sample stock solution: 100 mg/mL of Lithium Carbonate prepared as follows. Suspend 20.0 g of Lithium Carbonate in 100 mL of water, cautiously add 50.0 mL of hydrochloric acid, transfer to a 200-mL volumetric flask, and dilute with water to volume.

Standard solution: Transfer 1 mL of *Standard stock solution* and 5 mL of *Sample stock solution* to a 100-mL volumetric flask, and dilute with water.

Sample solution: 5 mg/mL of Lithium Carbonate from *Sample stock solution* diluted with water

Instrumental conditions

Mode: Flame photometry

Analytical wavelengths: 580 and 589 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Set the flame photometer for maximum emission at 589 nm, using the *Standard solution*. Measure the emission intensities of the *Sample solution* at 580 and 589 nm.

Acceptance criteria: The difference between the intensities observed at 580 and 589 nm for the *Sample solution* does not exceed the difference between the intensities observed at 589 nm for the *Sample solution* and the *Standard solution*, respectively (0.1%).

Delete the following:

• **HEAVY METALS (231)**

Sample solution: Dissolve 1 g of Lithium Carbonate in 10 mL of 3 N hydrochloric acid, and dilute with water to 25 mL.

Acceptance criteria: NMT 20 ppm • (Official 1-Jan-2018)

• **CHLORIDE AND SULFATE, Sulfate (221)**

Standard solution: Transfer 1 mL of 0.020 N sulfuric acid and 1 mL of 3 N hydrochloric acid to a suitable container. Dilute with water to 40 mL.

Sample solution: Transfer 1.0 g of Lithium Carbonate to a suitable container. Dissolve 10 mL of 3 N hydrochloric acid. Dilute with water to 40 mL.

Analysis: To the *Standard solution* and the *Sample solution*, separately, add 1 mL of barium chloride TS.

Acceptance criteria: The turbidity produced in the *Sample solution*, after 3 min, is NMT that produced in the *Standard solution* (0.1%).

• **CHLORIDE AND SULFATE, Chloride (221)**

Standard solution: Transfer 0.5 mL of 0.02 N hydrochloric acid and 1.2 mL of nitric acid to a suitable container. Dilute with water to 50 mL.

Sample solution: Transfer 500 mg of Lithium Carbonate to a suitable container. Add 1.2 mL of nitric acid. Dilute with water to 50 mL.

Analysis: To the *Standard solution* and the *Sample solution*, separately, add 1 mL of silver nitrate TS.

Acceptance criteria: The turbidity produced in the *Sample solution* is NMT that produced in the *Standard solution* (0.07%).

SPECIFIC TESTS

• **LOSS ON DRYING (731)**

Analysis: Dry at 200° for 4 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Lithium Carbonate Capsules

DEFINITION

Lithium Carbonate Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of lithium carbonate (Li_2CO_3).

IDENTIFICATION

- **A.** A portion of the Capsule contents effervesces upon the addition of an acid, yielding a colorless gas that, when passed into calcium hydroxide TS, immediately forms a white precipitate.
- **B.** The emission intensity at 671 nm of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the Assay.

ASSAY

• **PROCEDURE**

Surfactant solution: 1%–2% (v/v) solution of nonionic surfactant such as t-dodecyl mercaptan ethoxylate or polyoxyethylene (20) sorbitan monolaurate in water

Blank: *Surfactant solution* and water (1:50)

Standard stock solution: 0.3 mg/mL of USP Lithium Carbonate RS prepared as follows. Transfer the required quantity of USP Lithium Carbonate RS to a suitable volumetric flask, and add water to 20% of the flask volume and hydrochloric acid to 0.5% of the flask volume. Shake until dissolved, and dilute with water to volume.

Standard solution: 0.006 mg/mL of USP Lithium Carbonate RS from *Standard stock solution* prepared as follows. Pipet a suitable volume of *Standard stock solution* into a suitable volumetric flask. Add water to 80% of the flask volume and *Surfactant solution* to 2% of the flask volume, and dilute with water to volume.

Sample stock solution: Nominally 0.6 mg/mL of lithium carbonate from the contents of NLT 20 Capsules prepared as follows. Transfer a portion of powder, equivalent to NLT 600 mg of lithium carbonate, into a suitable volumetric flask. Add water to 4% of the flask volume and hydrochloric acid to 0.5% of the flask volume, shake until the solid is well disintegrated, and dilute with water to volume.

Sample solution: Nominally 0.006 mg/mL of lithium carbonate from *Sample stock solution* prepared as follows. Pipet a suitable volume of *Sample stock solution* into a suitable volumetric flask. Add water to 80% of the flask volume and *Surfactant solution* to 2% of the flask volume, and dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: About 671 nm

Analysis

Samples: *Blank*, *Standard solution*, and *Sample solution*
Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = emission response from the *Sample solution*
 r_S = emission response from the *Standard solution*
 C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS**• DISSOLUTION (711)**

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Surfactant solution, Blank, and Standard solution:Prepare as directed in the *Assay*.

Sample solution: Pass the solution under test through a suitable filter. Transfer 20.0 mL of the filtrate to a 1000-mL volumetric flask. Add 500 mL of water, 1 drop of hydrochloric acid, and 20 mL of *Surfactant solution*. Dilute with water to volume.

Analysis

Samples: *Blank*, *Standard solution*, and *Sample solution*
Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution* and *Sample solution*.

Calculate percentage of the labeled amount of lithium carbonate (Li_2CO_3) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (1/L) \times 100$$

r_U = emission response from the *Sample solution*
 r_S = emission response from the *Standard solution*
 C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)
 V = volume of the *Medium*, 900 mL
 D = dilution factor
 L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of lithium carbonate (Li_2CO_3) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements**ADDITIONAL REQUIREMENTS**

• PACKAGING AND STORAGE: Preserve in well-closed containers. Store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Lithium Carbonate RS

Lithium Carbonate Tablets**DEFINITION**

Lithium Carbonate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of lithium carbonate (Li_2CO_3).

IDENTIFICATION

A portion of the powdered Tablets meets the requirements of the following tests.

- **A.** It effervesces upon the addition of an acid, yielding a colorless gas that, when passed into calcium hydroxide TS, immediately forms a white precipitate.
- **B.** The emission intensity at 671 nm of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Surfactant solution: 1%–2% solution of nonionic surfactant such as t-dodecyl mercaptan ethoxylate or polyoxyethylene (20) sorbitan monolaurate in water

Blank: *Surfactant solution* and water (1:50)

Standard solution: Transfer 30 mg of USP Lithium Carbonate RS to a 100-mL volumetric flask, and add 20 mL of water and 0.5 mL of hydrochloric acid. Shake until dissolved, and dilute with water to volume. Pipet 20 mL of the resulting solution into a 1000-mL volumetric flask, add 800 mL of water and 20 mL of a suitable surfactant solution, and dilute with water to volume.

Sample solution: Powder NLT 20 Tablets. Transfer a portion of powder, nominally equivalent to 600 mg of lithium carbonate, into a 1000-mL volumetric flask. Add 40 mL of water and 5 mL of hydrochloric acid, shake until the solid is well disintegrated, and dilute with water to volume. Pipet 10 mL of the resulting solution into a 1000-mL volumetric flask, add 800 mL of water and 20 mL of the surfactant solution, and dilute with water to volume.

Instrumental conditions

Mode: Flame photometer

Analytical wavelength: About 671 nm

[NOTE—Adjust the instrument with the *Surfactant solution*.]**Analysis**

Samples: *Blank*, *Standard solution*, and *Sample solution*
Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)
 C_U = nominal concentration of lithium carbonate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS**• DISSOLUTION (711)**

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Surfactant solution, Blank, and Standard solution:Proceed as directed in the *Assay*.

Sample solution: Dilute 900 mL of the solution under test with *Medium* to 1000 mL. Pass through a suitable filter. Transfer 20.0 mL of the filtrate to a 1000-mL volumetric flask. Add 500 mL of water, 1 drop of hydrochloric acid, and 20 mL of *Surfactant solution*. Dilute with water to volume.

Instrumental conditions

Mode: Flame photometer

Analytical wavelength: About 671 nm

Analysis

Samples: *Blank*, *Standard solution*, and *Sample solution*
Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of lithium carbonate (Li_2CO_3) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from moisture. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Lithium Carbonate RS

Lithium Carbonate Extended-Release Tablets

DEFINITION

Lithium Carbonate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lithium carbonate (Li_2CO_3).

IDENTIFICATION

- **A.** A portion of powdered Tablets effervesces upon the addition of an acid, yielding a colorless gas that, when passed into calcium hydroxide TS, immediately forms a white precipitate.
- **B.** The emission intensity at 671 nm of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the Assay.

ASSAY

PROCEDURE

Diluent: Dilute 5 mL of hydrochloric acid with water to 1 L.

Standard solution: 0.3 mg/mL of USP Lithium Carbonate RS in *Diluent* prepared as follows. Transfer a suitable quantity of USP Lithium Carbonate RS to an appropriate volumetric flask. Add water to 20% of the flask volume, and hydrochloric acid to 0.5% of the flask volume.

Shake until dissolved, and dilute with water to volume.
Sample stock solution: Nominally 12 mg/mL of lithium carbonate from a number of Tablets, nominally equivalent to NLT 1200 mg of lithium carbonate prepared as follows. Transfer the required number of Tablets to a suitable container. Add the required amount of *Diluent*. Shake until completely dissolved. Filter, and discard the first 25 mL. Use the remaining filtrate.

Sample solution: Nominally 0.3 mg/mL from *Sample stock solution* in *Diluent*

[NOTE—The *Standard solution* and *Sample solution* may be diluted quantitatively with *Diluent*, if necessary, to yield solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: About 671 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Use the *Diluent* to zero the instrument. Measure the emission responses for the *Standard solution* and the *Sample solution*.

Calculate the percentage of labeled amount of lithium carbonate (Li_2CO_3) in the portion of Tablets:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times D \times 100$$

r_U = emission response from the *Sample solution*

r_S = emission response from the *Standard solution*

C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lithium carbonate in the *Sample solution* (mg/mL)

D = dilution factor, if needed

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Test 1

Medium: Dilute hydrochloric acid (7 in 1000); 800 mL

Apparatus 1: 100 rpm

Time: 15, 45, 90, and 120 min

Standard solution: USP Lithium Carbonate RS at a known concentration in *Medium*

Sample solution: At each time point i , withdraw 8.0 mL of the *Sample solution*, and pass through a filter of 35- μm or finer pore size. Use the filtrate as the *Sample solution*, suitably diluted with *Medium* if necessary.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: About 671 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Use the *Medium* to zero the instrument. Measure the emission responses for the *Standard solution* and the *Sample solution*.

Calculate the concentration, C_i , of lithium carbonate (Li_2CO_3) in *Medium* (mg/mL) at each time point i :

$$C_i = (r_U/r_S) \times C_S$$

r_U = emission response from the *Sample solution*

r_S = emission response from the *Standard solution*

C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount (Q) of lithium carbonate (Li_2CO_3) dissolved at each time point i :

$$\text{Result}_i = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_i = \{ \{ C_i \times [V - (i - 1) \times V_S] \} + [(C_{i-1} + C_{i-2} + \dots + C_1) \times V_S] \} \times (1/L) \times 100$$

C_i = concentration of lithium carbonate in *Medium* in the portion of sample withdrawn at time point i (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Capsule)

V_S = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See Table 1.

Table 1

Time Point (i)	Time (min)	Amount Dissolved (%)
1	15	2–16
2	45	25–45
3	90	60–85
4	120	NLT 85

The percentages of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution (711)*.

Test 2: If the product complies with this procedure, the labeling indicates that it meets *USP Dissolution Test 2*.

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 1, 3, and 7 h

Standard solution, Sample solution, Instrumental conditions, and Analysis: Proceed as directed in *Test 1*.

Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at each time point as described in *Test 1*.

Tolerances: See *Table 2*.

Table 2

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 40
2	3	45–75
3	7	NLT 70

The percentages of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* (711).

Test 3: If the product complies with this test, the labeling indicates that it meets *USP Dissolution Test 3*.

Medium: Water; 250 mL

Apparatus 3: 6 dips/min, 20-mesh top screen and 100-mesh bottom screen

Time: 1, 2, and 6 h

Standard solution, Sample solution, Instrumental conditions, and Analysis: Proceed as directed in *Test 1*.

Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at each time point as described in the *Test 1*.

Tolerances: See *Table 3*.

Table 3

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	10–45
2	2	25–75
3	6	NLT 70

The percentages of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* (711).

Test 4: If the product complies with this procedure, the labeling indicates that it meets *USP Dissolution Test 4*.

Medium: Dilute hydrochloric acid (7 in 1000); 800 mL

Apparatus 1: 100 rpm

Time: 15, 45, 90, and 120 min

Standard solution, Sample solution, Instrumental conditions, and Analysis: Proceed as directed in *Test 1*.

Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at each time point as described in the *Test 1*.

Tolerances: See *Table 4*.

Table 4

Time Point (i)	Time (min)	Amount Dissolved (%)
1	15	NMT 15
2	45	20–45
3	90	50–80
4	120	NLT 70

The percentages of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* (711).

Test 5: If the product complies with this procedure, the labeling indicates that it meets *USP Dissolution Test 5*.

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 30, 90, and 150 min

Standard solution, Sample solution, Instrumental conditions, and Analysis: Proceed as directed in *Test 1*.

Calculate the percentage of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at each time point as described in the *Test 1*.

Tolerances: See *Table 5*.

Table 5

Time Point (i)	Time (min)	Amount Dissolved (%)
1	30	10–30
2	90	55–75
3	150	NLT 85%

The percentages of the labeled amount of lithium carbonate (Li_2CO_3) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* (711).

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

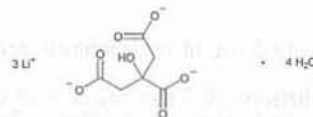
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Protect from moisture. Store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** (11)
USP Lithium Carbonate RS

Lithium Citrate



$\text{C}_6\text{H}_5\text{Li}_3\text{O}_7 \cdot 4\text{H}_2\text{O}$ 281.98

$\text{C}_6\text{H}_5\text{Li}_3\text{O}_7$ 209.93

1,2,3-Propanetricarboxylic acid, 2-hydroxy-trilithium salt tetrahydrate;

Trilithium citrate tetrahydrate [6080-58-6].

Anhydrous [919-16-4].

DEFINITION

Lithium Citrate contains NLT 98.0% and NMT 102.0% of lithium citrate ($\text{C}_6\text{H}_5\text{Li}_3\text{O}_7$), calculated on the anhydrous basis.

IDENTIFICATION

• **A.** The emission intensity at 671 nm of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. IDENTIFICATION TESTS—GENERAL, Citrate** (191): Meets the requirement

ASSAY

• PROCEDURE

Surfactant solution: 1%–2% solution of nonionic surfactant such as t-dodecyl mercaptan ethoxylate or polyoxyethylene (20) sorbitan monolaurate in water

Blank: *Surfactant solution* and water (1:50)

Standard stock solution: 0.3 mg/mL of USP Lithium Carbonate RS prepared as follows. Transfer the required quantity of USP Lithium Carbonate RS to a suitable volumetric flask, and add 20% of the flask volume of water and 0.5% of the flask volume of hydrochloric acid. Shake until dissolved, and dilute with water to volume.

Standard solution: 0.006 mg/mL of USP Lithium Carbonate RS from *Standard stock solution* prepared as fol-

lows. Pipet a suitable volume of *Standard stock solution* into a suitable volumetric flask. Add 80% of the flask volume of water and 2% of the flask volume of the *Surfactant solution*, and dilute with water to volume.

Sample stock solution: 0.8 mg/mL of Lithium Citrate prepared as follows. Transfer a suitable amount of Lithium Citrate to a suitable volumetric flask. Dissolve in water. Add 0.05% of the flask volume of hydrochloric acid. Dilute with water to volume.

Sample solution: Nominally 0.006 mg/mL of Lithium Citrate from *Sample stock solution* prepared as follows. Pipet a suitable volume of *Sample stock solution* into a suitable volumetric flask. Add 80% of the flask volume of water and 2% of the flask volume of the *Surfactant solution*, and dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: 671 nm

Analysis

Samples: *Blank*, *Standard solution*, and *Sample solution*. Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution* and the *Sample solution*.

Calculate the percentage of lithium citrate ($C_6H_5Li_3O_7$) in the portion of Lithium Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times F \times 100$$

r_U = emission response of the *Sample solution*

r_S = emission response of the *Standard solution*

C_S = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)

C_U = concentration of Lithium Citrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of anhydrous lithium citrate, 209.93

M_{r2} = molecular weight of lithium carbonate, 73.89

F = ratio of the number of moles of lithium in 1 mol of lithium carbonate to that of the number of moles of lithium in 1 mol of lithium citrate, 0.66

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Delete the following:

• HEAVY METALS (231)

Test preparation: Dissolve 2.0 g of Lithium Citrate in 2 mL of 0.1 N hydrochloric acid, and dilute with water to 25 mL.

Acceptance criteria: NMT 10 ppm • (Official 1-Jan-2018)

SPECIFIC TESTS

• CARBONATE

Analysis: Add 0.5 g of Lithium Citrate to 5 mL of 6 N acetic acid.

Acceptance criteria: NMT a slight effervescence is produced.

• PH (791)

Sample solution: 50-mg/mL solution of Lithium Citrate in water

Acceptance criteria: 7.0–10.0

• WATER DETERMINATION, Method III (921)

Analysis: Dry a sample at 150° for 3 h.

Acceptance criteria: 24.0%–28.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Lithium Carbonate RS

Lithium Hydroxide

LiOH · H₂O

41.96

LiOH

23.95

Lithium hydroxide monohydrate [1310-66-3].

Anhydrous [1310-65-2].

DEFINITION

Lithium Hydroxide contains NLT 98.0% and NMT 102.0% of lithium hydroxide (LiOH), calculated on the anhydrous basis.

[CAUTION]—Exercise great care in handling Lithium Hydroxide, as it rapidly destroys tissues.]

IDENTIFICATION

• **A.** When moistened with hydrochloric acid, it imparts an intense crimson color to a nonluminous flame.

ASSAY

• PROCEDURE

Sample solution: Nominally equivalent to 10 mg/mL of anhydrous lithium hydroxide from Lithium Hydroxide in carbon dioxide-free water

Analysis

Preliminary titration: Pipet 50 mL of the *Sample solution* into a 250-mL conical flask. Start the titration by adding 35 mL of 0.5 N hydrochloric acid VS with continuous vigorous stirring. Add 20 mL of 1 N barium chloride and 3 drops of phenolphthalein TS, and allow to stand for 2 min. Continue the titration with 0.5 N hydrochloric acid VS. At the discharge of the pink color of the indicator, record the volume of acid solution consumed.

Final titration: Pipet 50 mL of the *Sample solution* into a 250-mL conical flask. While pipeting and during the subsequent titrations, keep the contents of the flask blanketed with a stream of carbon dioxide-free air. Start the titration by adding with continuous vigorous swirling a volume of 0.5 N hydrochloric acid VS that is 0.50 mL less than that consumed in the preliminary titration. Add 20 mL of 1 N barium chloride and 3 drops of phenolphthalein TS, and allow to stand for 2 min. Rinse the sides of the flask with carbon dioxide-free water, and continue the titration with 0.1 N hydrochloric acid VS. At the discharge of the pink color of the indicator, record the volume of acid solution consumed. Each mL of 0.5 N hydrochloric acid VS and 0.1 N hydrochloric acid VS is equivalent to 11.975 and 2.395 mg of total alkali, respectively, calculated as lithium hydroxide (LiOH).

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• CHLORIDE AND SULFATE, Sulfate (221)

Sample: 2.0 g

Acceptance criteria: It shows no more sulfate than corresponds to 1.0 mL of 0.020 N sulfuric acid (0.05%).

• CALCIUM

Sample solution: Dissolve 3.33 g in 50 mL of 3 N hydrochloric acid. Boil the clear solution to expel carbon dioxide, add 5 mL of ammonium oxalate TS, render al-

kaline with 6 N ammonium hydroxide, and allow to stand for 4 h. Pass through a filtering crucible, and wash with warm water until the last washing yields no turbidity with calcium chloride TS. Place the crucible in a beaker, cover it with water, add 3 mL of sulfuric acid, and heat to 70°.

Analysis: Titrate the *Sample solution* with 0.10 N potassium permanganate to a pale pink color that persists for 30 s.

Acceptance criteria: NMT 3.34 mL of 0.10 N potassium permanganate is consumed (0.20%).

• CARBONATE

[NOTE—While pipeting and during the subsequent titrations, keep the contents of the flasks blanketed with a stream of carbon dioxide-free air.]

Analysis: To the flask containing the completed *Final titration* obtained in the *Assay*, add 1 drop of methyl orange TS. Titrate with 0.1 N hydrochloric acid VS until a persistent orange color is produced and no undissolved barium carbonate remains. Perform a blank titration to determine the volume of 0.1 N hydrochloric acid consumed in going from the phenolphthalein endpoint to the methyl orange endpoint. To 100 mL of carbon dioxide-free water in a 250-mL conical flask, add 3 drops of the *Sample solution* from the *Assay*, 20 mL of 1 N barium chloride, and 3 drops of phenolphthalein TS. Allow to stand for 2 min. Titrate this solution with 0.1 N hydrochloric acid. At the discharge of the pink color of the indicator, add 1 drop of methyl orange TS, and titrate with 0.1 N hydrochloric acid VS until a persistent orange color is produced.

Acceptance criteria: The titration shows no more carbon dioxide than corresponds to 1.5 mL of 0.10 N hydrochloric acid (0.7%).

Delete the following:

• HEAVY METALS, Method I (231)

Sample solution: Dissolve 1.0 g in 15 mL of 3 N hydrochloric acid, and dilute with water to 25 mL.

Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

SPECIFIC TESTS

• LITHIUM CONTENT

Standard stock solution: 0.3 mg/mL of USP Lithium Carbonate RS prepared as follows. Dissolve first in water using 20% final volume and hydrochloric acid using 0.5% of final volume. Dilute with water to volume.

Standard solution: 6.0 µg/mL of USP Lithium Carbonate RS from the *Standard stock solution* prepared as follows. Pipet a volume of the *Standard stock solution* into a suitable volumetric flask, add water to fill 80% of final volume, and a suitable surfactant solution to fill 2% of final volume. Dilute with water to volume. Measure the pH.

Sample stock solution: 0.4 mg/mL of Lithium Hydroxide in water

Sample solution: Pipet 20 mL of the *Sample stock solution* into a 1000-mL volumetric flask. Add 950 mL of water, 2 mL of 1 N hydrochloric acid, and 20 mL of a surfactant solution, and mix. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to the same pH (±0.1 pH unit) as that of the *Standard solution*, and dilute with water to volume.

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: 671 nm. Adjust the instrument with the surfactant solution.

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lithium (Li) in the portion of Lithium Hydroxide taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (A_r/M_r) \times F \times 100$$

r_u = photometric reading of the *Sample solution*
 r_s = photometric reading of the *Standard solution*
 C_s = concentration of USP Lithium Carbonate RS in the *Standard solution* (mg/mL)
 C_u = concentration of Lithium Hydroxide in the *Sample solution* (mg/mL)
 A_r = atomic weight of lithium, 6.94
 M_r = molecular weight of lithium carbonate, 73.89
 F = number of lithium ions per mole of lithium carbonate, 2

Acceptance criteria: 28.1%–29.9% on the anhydrous basis

• WATER DETERMINATION, Method III (921)

Analysis: Dry at 135° at a pressure of NMT 5 mm of mercury for 1 h.

Acceptance criteria: 41.0%–43.5%

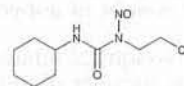
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Lithium Carbonate RS

Lomustine



$C_9H_{16}ClN_3O_2$ 233.70
 Urea, N-(2-chloroethyl)-N'-cyclohexyl-N-nitroso-;
 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea [13010-47-4].

DEFINITION

Lomustine contains NLT 97% and NMT 103% of lomustine ($C_9H_{16}ClN_3O_2$), calculated on the as-is basis.

[CAUTION—Great care should be taken to prevent inhalation of particles of lomustine and to prevent exposure to the skin.]

IDENTIFICATION

• **A. INFRARED ABSORPTION (197K)**

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

[NOTE—Protect the solutions from light. Use freshly prepared solutions.]

Mobile phase: Acetonitrile and water (35:65)

Standard solution: 0.2 mg/mL of USP Lomustine RS in acetonitrile

Sample solution: 0.2 mg/mL of Lomustine in acetonitrile

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 230 nm**Column:** 4.6-mm × 75-mm; 3-μm packing L1**Flow rate:** 1.5 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 1.0%**Tailing factor:** NMT 1.3**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of lomustine ($C_9H_{16}ClN_3O_2$) in the portion of Lomustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Lomustine RS in the *Standard solution* (mg/mL) C_U = concentration of Lomustine in the *Sample solution* (mg/mL)**Acceptance criteria:** 97%–103% on the as-is basis**IMPURITIES****Delete the following:**• **HEAVY METALS****Magnesium sulfate solution:** 250 mg/mL of magnesium sulfate in 2 N sulfuric acid**Lead nitrate stock solution and Standard lead solution:** Prepare as directed in *Heavy Metals* (231), *Special Reagents*.**Standard solution:** Transfer 4.0 mL of *Standard lead solution* into a 50-mL color-comparison tube, and dilute with water to 25 mL.

Sample solution: Transfer 2.0 g of Lomustine to a 50-mL silica crucible. Add 4 mL of *Magnesium sulfate solution*, and mix using a thin glass rod. Place the crucible on a steam bath, and heat cautiously until charring begins. Then place the crucible on a hot plate, and continue the charring. When the charring is complete, carry out the ignition at a temperature not exceeding 800° until an almost white or a mostly grayish residue is obtained. Allow to cool, and moisten the residue with 0.5 mL of 2 N hydrochloric acid, evaporate, ignite again, and allow to cool. The total period of ignition must not exceed 2 h. Dissolve the residue in two portions, 5 mL each, of 2 N hydrochloric acid. Add 0.1 mL of phenolphthalein TS, followed by ammonium hydroxide, until a pink color is obtained. Cool, add glacial acetic acid until the solution is decolorized, then add an additional 0.5 mL of glacial acetic acid. Filter if necessary, dilute with water to 20 mL, and quantitatively transfer this solution into a 50-mL color-comparison tube.

Analysis**Samples:** *Standard solution* and *Sample solution*

Adjust with either 1 N acetic acid or 6 N ammonium hydroxide to a pH of between 3.0 and 4.0 using litmus paper. Dilute with water to 40 mL. Add 1.2 mL of thioacetamide–glycerin base TS, and dilute with water to volume. Allow to stand for 2 min, and view downward over a white surface.

Acceptance criteria: NMT 20 μg/g; any brown color in the *Sample solution* is not more intense than that in the *Standard solution*. • (Official 1-Jan-2018)• **ORGANIC IMPURITIES**

[NOTE—Protect the solutions from light. Use freshly prepared solutions.]

Solution A: Water**Solution B:** Acetonitrile**Mobile phase:** See Table 1.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3	90	10
40	65	35
47	65	35
52	50	50
55	50	50
55.1	5	95
60	5	95
60.1	90	10
65	90	10

Standard solution A: 0.032 mg/mL each of USP Carmustine Related Compound A RS, USP Lomustine Related Compound B RS, and USP Lomustine Related Compound C RS, and 0.04 mg/mL of USP Lomustine Related Compound D RS, and 2 mg/mL of USP Lomustine RS in acetonitrile

Standard solution B: 8 μg/mL of USP Lomustine RS in acetonitrile

Sample solution: 8 mg/mL of Lomustine in acetonitrile

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 195 and 230 nm**Column:** 4.6-mm × 10-cm; 2.6-μm packing L43**Temperatures****Column:** 35°**Sample:** 15°**Flow rate:** 0.8 mL/min**Injection volume:** 4 μL**System suitability****Samples:** *Standard solution A* and *Standard solution B***Suitability requirements****Resolution:** NLT 1.2 between the lomustine and lomustine related compound D peaks determined at 230 nm, *Standard solution A*

Relative standard deviation: NMT 10% for carmustine related compound A and lomustine related compounds B and C determined at 195 nm, *Standard solution A*; NMT 10% for lomustine related compound D determined at 230 nm, *Standard solution A*; NMT 10% for lomustine determined at 230 nm, *Standard solution B*

Tailing factor: Between 0.7 and 1.3 for the lomustine peak determined at 230 nm, *Standard solution A*

Analysis**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of carmustine related compound A and lomustine related compounds B and C in the portion of Lomustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak area of each impurity from the *Sample solution*, determined at 195 nm r_S = peak area of the corresponding related compound from *Standard solution A*, determined at 195 nm

C_S = concentration of the corresponding USP Carmustine Related Compound A RS, USP Lomustine Related Compound B RS, or USP Lomustine Related Compound C RS in *Standard solution A* (mg/mL)

 C_U = concentration of Lomustine in the *Sample solution* (mg/mL)

Detect at 230 nm, and compare the peak area of lomustine related compound D in the *Sample solution* with the peak area of lomustine related compound D in *Standard solution A*. The peak area of lomustine related compound D in the *Sample solution* is NMT the peak area of lomustine related compound D in *Standard solution A* (0.5%).

Calculate the percentage of any unspecified impurity in the portion of Lomustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of any unspecified impurity from the *Sample solution*, determined at 195 nm or 230 nm. If the impurity is detected at both wavelengths, use the higher peak area in the formula.

r_S = peak area of lomustine from *Standard solution B*, determined at 230 nm

C_S = concentration of USP Lomustine RS in *Standard solution B* (mg/mL)

C_U = concentration of Lomustine in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carmustine related compound A ^a	0.11	0.4 ^b
Lomustine related compound B ^c	0.39	0.4 ^b
Lomustine related compound C ^d	0.73	0.4 ^b
Lomustine	1.0	—
Lomustine related compound D ^e	1.02	0.5
Any individual unspecified impurity	—	0.2
Total impurities ^f	—	1

^a 1,3-Bis(2-chloroethyl)urea.

^b No more than one such impurity (carmustine related compound A, lomustine related compound B, or lomustine related compound C) is greater than 0.2%.

^c 1-(2-Chloroethyl)-3-cyclohexylurea.

^d 1,3-Dicyclohexylurea.

^e 3-(2-Chloroethyl)-1-cyclohexyl-1-nitrosourea.

^f Lomustine related compound D is not included in the *Total impurities*.

SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store between 2° and 8°.

- **USP REFERENCE STANDARDS (11)**

USP Carmustine Related Compound A RS

1,3-Bis(2-chloroethyl)urea.

$C_5H_{10}Cl_2N_2O$ 185.05

USP Lomustine RS

USP Lomustine Related Compound B RS

1-(2-Chloroethyl)-3-cyclohexylurea.

$C_9H_{17}ClN_2O$ 204.70

USP Lomustine Related Compound C RS

1,3-Dicyclohexylurea.

$C_{13}H_{24}N_2O$ 224.34

USP Lomustine Related Compound D RS

3-(2-Chloroethyl)-1-cyclohexyl-1-nitrosourea.

$C_9H_{16}ClN_3O_2$ 233.70

Lomustine Capsules

DEFINITION

Lomustine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of lomustine ($C_9H_{16}ClN_3O_2$).

[CAUTION]—Great care should be taken to prevent inhalation of particles of lomustine and to prevent exposure to the skin.]

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

Sample: Transfer the contents of 2 Capsules to a stoppered Erlenmeyer flask containing 25 mL of methylene chloride. Shake vigorously, and filter. Evaporate the methylene chloride from the filtrate under a stream of dry nitrogen. Prepare a potassium bromide pellet of the residue.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

- **PROCEDURE**

[NOTE—Protect the solutions from light. Use freshly prepared solutions.]

Mobile phase: Acetonitrile and water (7:13)

Standard solution: 0.2 mg/mL of USP Lomustine RS in acetonitrile

Sample solution: 0.2 mg/mL of lomustine in acetonitrile prepared as follows. Transfer a portion of the Capsule fill (from NLT 20 Capsules) to a suitable volumetric flask, and add acetonitrile equivalent to 75% of the volume. Shake for 15 min, and dilute with acetonitrile to volume. Pass through a suitable filter, and discard the first few mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 7.5-cm; 3-μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Tailing factor: NMT 1.3

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lomustine ($C_9H_{16}ClN_3O_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of lomustine in the *Standard solution* (mg/mL)

C_U = nominal concentration of lomustine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

- **ORGANIC IMPURITIES**

[NOTE—Protect the solutions from light. Use freshly prepared solutions.]

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3	90	10
40	65	35
47	65	35
52	50	50
55	50	50
55.1	5	95
60	5	95
60.1	90	10
65	90	10

Standard solution A: 0.032 mg/mL each of USP Carmustine Related Compound A RS, USP Lomustine Related Compound B RS, and USP Lomustine Related Compound C RS, and 0.04 mg/mL of USP Lomustine Related Compound D RS and 2 mg/mL of USP Lomustine RS in acetonitrile

Standard solution B: 8 µg/mL of USP Lomustine RS in acetonitrile

Sample solution: 8 mg/mL of lomustine in acetonitrile prepared as follows. Transfer a portion of the Capsule fill (from NLT 20 Capsules) to a suitable volumetric flask, and dilute with acetonitrile to volume. Sonicate for 30 min, and then stir for 30 min. Pass a portion of the solution through a suitable filter of 0.2-µm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 195 and 230 nm

Column: 4.6-mm × 10-cm; 2.6-µm packing L43

Temperatures

Column: 35°

Sample: 15°

Flow rate: 0.8 mL/min

Injection volume: 4 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Resolution: NLT 1.2 between the lomustine and lomustine related compound D peaks determined at 230 nm, *Standard solution A*

Relative standard deviation: NMT 10% for carmustine related compound A, lomustine related compounds B and C determined at 195 nm, *Standard solution A*; NMT 10% for lomustine determined at 230 nm, *Standard solution B*

Tailing factor: Between 0.7 and 1.3 for the lomustine peak determined at 230 nm, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of carmustine related compound A and lomustine related compounds B and C in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*, determined at 195 nm

r_S = peak area of the corresponding related compound from *Standard solution A*, determined at 195 nm

C_S = concentration of the corresponding USP Carmustine Related Compound A RS, USP Lomustine Related Compound B RS, or USP Lomustine Related Compound C RS in *Standard solution A* (mg/mL)

C_U = nominal concentration of lomustine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of any unspecified impurity from the *Sample solution*, determined at 195 nm or 230 nm. If the impurity is detected at both wavelengths, use the higher peak area in the formula.

r_S = peak area of lomustine from *Standard solution B*, determined at 230 nm

C_S = concentration of USP Lomustine RS in the *Standard solution B* (mg/mL)

C_U = nominal concentration of lomustine in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carmustine related compound A ^a	0.11	0.4 ^b
Lomustine related compound B ^c	0.39	0.4 ^b
Lomustine related compound C ^d	0.73	0.4 ^b
Lomustine	1.0	—
Lomustine related compound D ^e	1.02	—
Any individual unspecified impurity	—	0.2
Total impurities	—	2

^a 1,3-Bis(2-chloroethyl)urea.

^b No more than one such impurity (carmustine related compound A, lomustine related compound B, or lomustine related compound C) is greater than 0.2%.

^c 1-(2-Chloroethyl)-3-cyclohexylurea.

^d 1,3-Dicyclohexylurea.

^e This process impurity is included in the table for identification only, and it is not to be reported or included in the *Total impurities*.

PERFORMANCE TESTS

• **DISINTEGRATION** (701): 20 min

• **UNIFORMITY OF DOSAGE UNITS** (905)

Procedure for content uniformity

Complete the analysis within 1 h after the preparation of *Sample solution*.

Standard solution: 0.02 mg/mL of USP Lomustine RS in methanol

Sample solution: Nominally equivalent to 0.02 mg/mL of lomustine in methanol prepared as follows. Transfer the content of a Capsule into a 100-mL volumetric flask, and dilute with methanol to volume. Allow the excipients to settle for at least 15 min. Transfer a measured volume of the clear supernatant to a suitable volumetric flask, and dilute to volume with methanol.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 230 nm

Cell length: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lomustine (C₉H₁₆ClN₃O₂) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

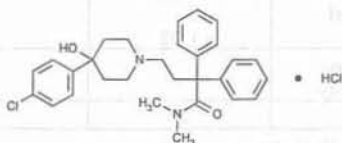
- A_U = absorbance from the *Sample solution*
 A_S = absorbance from the *Standard solution*
 C_S = concentration of USP Lomustine RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of lomustine in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
 - USP Carmustine Related Compound A RS
1,3-Bis(2-chloroethyl)urea.
 $C_5H_{10}Cl_2N_2O$ 185.05
 - USP Lomustine RS
 - USP Lomustine Related Compound B RS
1-(2-Chloroethyl)-3-cyclohexylurea.
 $C_9H_{17}ClN_2O$ 204.70
 - USP Lomustine Related Compound C RS
1,3-Dicyclohexylurea.
 $C_{13}H_{24}N_2O$ 224.34
 - USP Lomustine Related Compound D RS
3-(2-Chloroethyl)-1-cyclohexyl-1-nitrosourea.
 $C_9H_{16}ClN_3O_2$ 233.70

Loperamide Hydrochloride



$C_{29}H_{33}ClN_2O_2 \cdot HCl$ 513.50

1-Piperidinebutanamide, 4-(4-chlorophenyl)-4-hydroxy-*N,N*-dimethyl- α,α -diphenyl-, monohydrochloride.

4-(*p*-Chlorophenyl)-4-hydroxy-*N,N*-dimethyl- α,α -diphenyl-1-piperidinebutanamide monohydrochloride [34552-83-5].

» Loperamide Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{29}H_{33}ClN_2O_2 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Loperamide Hydrochloride RS

Identification—

A: *Infrared Absorption* (197K).

B: Transfer about 40 mg, accurately weighed, to a 100-mL volumetric flask, dissolve in about 50 mL of isopropyl alcohol, add 10 mL of 0.1 N hydrochloric acid, dilute with isopropyl alcohol to volume, and mix: the UV absorption spectrum between 250 and 300 nm of this solution exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Loperamide Hydrochloride RS, concomitantly measured.

Loss on drying (731)—Dry it in vacuum at 80° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.2%.

Delete the following:

• **Heavy metals, Method II** (231): 0.002%. • (Official 1-Jan-2018)

Chromatographic purity—Prepare a test solution in chloroform containing 10 mg per mL. Apply 10 μ L of this solution and 10 μ L of a Standard solution of USP Loperamide Hydrochloride RS in chloroform containing 10 mg per mL to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and formic acid (85:10:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Locate the spots on the plate by exposing it to fumes of iodine: the spot obtained from the test solution corresponds in R_f value, color, and intensity to that obtained from the Standard solution, and no secondary spots are observed.

Chloride content—Using about 13 mg, accurately weighed, proceed as directed under *Oxygen Flask Combustion* (471), using a mixture of 10 mL of 0.02 N sodium hydroxide and 2 drops of 30 percent hydrogen peroxide as the absorbing liquid. When combustion is complete and the combustion gases absorbed, rinse the stopper, sample holder, and inner walls of the flask with 50 mL of isopropyl alcohol. Add 4 mL of 0.1 N nitric acid, and titrate with 0.01 N mercuric nitrate VS, using diphenylcarbazone TS as the indicator. Each mL of 0.01 N mercuric nitrate is equivalent to 0.3545 mg of chlorine: between 13.52% and 14.20% is found.

Assay—

Neutralized acetic acid—Dissolve 10 mg of α -naphtholbenzein in 100 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid to a green endpoint, disregarding the amount of titrant consumed.

Procedure—Dissolve about 375 mg, accurately weighed, of Loperamide Hydrochloride in 25 mL of *Neutralized acetic acid*. Add 10 mL of mercuric acetate solution (prepared by dissolving 1 g of mercuric acetate in 33 mL of *Neutralized acetic acid*) and titrate with 0.1 N perchloric acid VS to the original green color of the *Neutralized acetic acid*. Each mL of 0.1 N perchloric acid is equivalent to 51.35 mg of $C_{29}H_{33}ClN_2O_2 \cdot HCl$.

Loperamide Hydrochloride Capsules

» Loperamide Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Loperamide Hydrochloride RS

Identification—

A: *Thin-Layer Chromatographic Identification Test* (201)—

Test solution—Transfer a quantity of the contents of the Capsules, equivalent to about 10 mg of loperamide hydrochloride, to a 37-mL stoppered vial, add 10 mL of methanol, shake for 5 minutes, and filter.

Standard solution: a solution of USP Loperamide Hydrochloride RS in methanol containing about 10 mg per mL.

Application volume: 10 μ L of the *Test solution* and 1 μ L of the *Standard solution*.

Developing solvent system: a mixture of chloroform, methanol, and formic acid (85:10:5).

Procedure—Proceed as directed in the chapter. Visualize the spots by exposing to iodine vapors.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)—

Medium: pH 4.7 acetate buffer, prepared by mixing 200 mL of 1 N acetic acid with 600 mL of water, adjusting with 1 N sodium hydroxide to a pH of 4.70 ± 0.05 , diluting with water to 1000 mL, and mixing; 500 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Determine the amount of loperamide hydrochloride dissolved using the following method.

Mobile phase and Chromatographic system—Proceed as directed in the *Assay*.

Procedure—Inject a volume (about 50 μ L) of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the quantity of $C_{29}H_{33}ClN_2O_2 \cdot HCl$ dissolved in comparison with a *Standard solution* having a known concentration of USP Loperamide Hydrochloride RS in the same medium and similarly chromatographed.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{29}H_{33}ClN_2O_2 \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase—Transfer 500 mL of acetonitrile to a 1000-mL volumetric flask. Dilute with water to volume, add 20 drops of phosphoric acid, mix, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Loperamide Hydrochloride RS in a mixture of acetonitrile and 0.5 N hydrochloric acid (1:1) to obtain a solution having a known concentration of about 0.2 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with a mixture of acetonitrile and water (1:1) to volume, and mix to obtain a solution having a known concentration of about 10 μ g per mL.

Assay preparation—Transfer, as completely as possible, the contents of not less than 20 Capsules to a suitable tared container, and determine the average weight per capsule. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 20 mg of loperamide hydrochloride, to a 100-mL volumetric flask. Add about 35 mL of 0.5 N hydrochloric acid and sonicate for 15 minutes. Add 35 mL of acetonitrile and sonicate for an additional 15 minutes. Dilute with a mixture of acetonitrile and 0.5 N hydrochloric acid (1:1) to volume, mix, and filter. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with a mixture of acetonitrile and water (1:1) to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4-mm \times 25-cm column that contains 10- μ m packing L10. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency, N , determined from the analyte peak is not less than 1900 theoretical plates, the capacity factor, K' , is not less than 3.5, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{29}H_{33}ClN_2O_2 \cdot HCl$ in the portion of Capsules taken by the formula:

$$2000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Loperamide Hydrochloride RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Loperamide Hydrochloride Oral Solution

DEFINITION

Loperamide Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

Sample: Transfer a volume of Oral Solution containing a suitable amount of loperamide hydrochloride (typically 12–24 mg) to a separator containing about 100 mL of water and 1 mL of 50% sodium hydroxide solution, and gently swirl the contents. Add 50 mL of methylene chloride, shake gently by hand, releasing pressure often, and then shake by mechanical means for 20 min. Allow the layers to separate. Transfer the lower methylene chloride layer to a separator containing 100 mL of water. Shake gently by hand, releasing pressure often, and then shake by mechanical means for 10 min. Allow the layers to separate. Transfer the lower methylene chloride layer to a 250-mL beaker, and evaporate to dryness on a steam bath with the aid of a current of air. Add 10 mL of methanol and 500 mg of potassium bromide to the beaker. Evaporate to dryness on a steam bath with the aid of a current of air, and use the residue.

Acceptance criteria: The spectrum obtained from the *Sample* shows bands at approximately 3400 cm^{-1} , 2929 cm^{-1} , 1624 cm^{-1} , and 1493 cm^{-1} , similar to the spectrum from a *Standard* similarly obtained.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer: 3.0 g/L of monobasic potassium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (37:63), adjusted with 5% phosphoric acid to a pH of 3.0

Standard stock solution: Prepare 2 mg/mL of USP Loperamide Hydrochloride RS in methanol. Dilute this solution with water to obtain a 0.1-mg/mL solution.

Standard solution: 0.01 mg/mL of USP Loperamide Hydrochloride RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Nominally 0.01 mg/mL of loperamide hydrochloride from Oral Solution, prepared as follows. Transfer a volume of Oral Solution, equivalent to about 1.0 mg of loperamide hydrochloride, to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, mix, and filter.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 214 nm**Column:** 4.0-mm × 8.0-cm; 5-μm packing L7, 4.6-mm × 7.5-cm; 3.5-μm packing L7, or 4.6-mm × 12.5-cm; 5-μm packing L7**Flow rate:** 1.5 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak area from the *Sample solution* r_S = peak area from the *Standard solution* C_S = concentration of USP Loperamide Hydrochloride RS in the *Standard solution* (mg/mL) C_U = nominal concentration of loperamide hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**OTHER COMPONENTS**

- ALCOHOL DETERMINATION, Method II (611)** (if present): 90.0%–110.0% of the labeled amount of alcohol (C_2H_5OH)

PERFORMANCE TESTS

- UNIFORMITY OF DOSAGE UNITS (905)**
For single-unit containers
Acceptance criteria: Meets the requirements
- DELIVERABLE VOLUME (698)**
For multiple-unit containers
Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- PH (791):** 2.7–6.5

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store below 40°, preferably between 15° and 30°, unless otherwise specified by the manufacturer.
- USP REFERENCE STANDARDS (11)**
USP Loperamide Hydrochloride RS

Loperamide Hydrochloride Tablets**DEFINITION**Loperamide Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$).**IDENTIFICATION**

- A. ULTRAVIOLET ABSORPTION (197U)**
[NOTE—This procedure is not applicable for Tablets labeled as chewable.]
Wavelength range: 250–300 nm
Standard solution: About 0.4 mg/mL of USP Loperamide Hydrochloride RS, prepared as follows. Transfer an amount of USP Loperamide Hydrochloride RS to a suitable volumetric flask, dissolve first in isopropyl alcohol, using 50% of the final volume. Add 0.1 N hydro-

chloric acid equivalent to 10% of the final volume, and dilute with isopropyl alcohol to volume.

Sample solution: Transfer a quantity of finely powdered Tablets equivalent to about 10 mg of loperamide hydrochloride to a test tube. Add 20.0 mL of isopropyl alcohol, shake by mechanical means for 1 min, and allow to settle. Pipet 9.0 mL of the supernatant into a 10-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.**Acceptance criteria:** The spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, concomitantly measured.**THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

[NOTE—For Tablets labeled as chewable, use the following procedure.]

Standard solution: 1.0 mg/mL of USP Loperamide Hydrochloride RS in methanol**Sample solution:** Grind a number of Tablets, equivalent to 10 mg of loperamide hydrochloride, with 10 mL of methanol for about 2 min. Centrifuge the mixture, and use the supernatant.**Application volume:** 10 μL**Developing solvent system:** Chloroform, methanol, and formic acid (75:25:1)**Analysis:** Visualize the spots by using Dragendorff's TS.**Acceptance criteria:** Meet the requirements

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY**PROCEDURE****Solvent mixture:** Methanol and acetonitrile (3:1)**Ion pairing solution:** Solution containing 2.35 g/L of sodium 1-hexanesulfonate and 2.88 g/L of monobasic ammonium phosphate in water, adjusted with phosphoric acid to a pH of 3.2**Mobile phase:** *Solvent mixture* and *Ion pairing solution* (55:45)**System suitability solution:** 0.2 mg/mL of USP Loperamide Hydrochloride RS and 0.002 mg/mL of USP Loperamide Related Compound F RS in *Mobile phase***Standard solution:** 0.2 mg/mL of USP Loperamide Hydrochloride RS in *Mobile phase***Sample solution:** Fill a 100-mL volumetric flask with *Mobile phase*. Immediately transfer a number of Tablets equivalent to 20 mg of loperamide hydrochloride to the flask, and cap tightly. Sonicate for 15–30 min with intermittent shaking. Allow the contents to settle, and use a clear supernatant.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 219 nm**Column:** 3.9-mm × 15-cm; 5-μm or 10-μm packing L1**Flow rate:** 2 mL/min**Injection volume:** 50 μL**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 3.0 between loperamide and loperamide related compound F, *System suitability solution***Tailing factor:** NMT 2.0 for both peaks, *System suitability solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Loperamide Hydrochloride RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of loperamide hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Loperamide Hydrochloride RS at a known concentration in *Medium*. [NOTE—If necessary, dissolve USP Loperamide Hydrochloride RS in a minimal amount of methanol, and then dilute with *Medium* to final concentration.]

Sample solution: Filtered solution under test

Buffer: Transfer 3.0 g of triethylamine hydrochloride and 1.0 mL of phosphoric acid to a 1-L flask, and add 550 mL of water.

Mobile phase: Acetonitrile and Buffer (45:55)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 7.5-cm; 3.5-μm packing L7 or 4.6-mm × 12.5-cm; 5-μm packing L7

Flow rate: 1.5 mL/min

Injection volume: 50 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Solvent mixture, Ion pairing solution, Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 0.002 mg/mL of USP Loperamide Related Compound F RS in *Mobile phase*

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 3.0 between loperamide and loperamide related compound F

Tailing factor: NMT 2.0 for both peaks

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of loperamide N-oxide in the portion of Tablets taken:

$$\text{Result} = (r_T/r_S) \times (C_S/C_U) \times 100$$

r_T = sum of the peak responses of the *cis* and *trans* isomers of N-oxide from the *Sample solution*

r_S = peak response of loperamide related compound F from the *Standard solution*

C_S = concentration of USP Loperamide Related Compound F RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of loperamide hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Loperamide	1.0	—
Loperamide <i>trans</i> -N-oxide (loperamide related compound F)	1.5	2.0 ^a
Loperamide <i>cis</i> -N-oxide ^b	1.7	

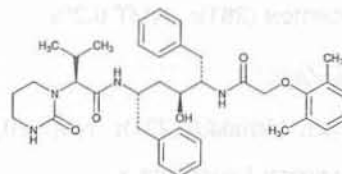
^a For the sum of *trans* and *cis*-isomers.

^b (1*S*,4*R*)-4-(4-Chlorophenyl)-1-[4-(dimethylamino)-4-oxo-3,3-diphenylbutyl]-4-hydroxypiperidine 1-oxide.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **LABELING:** Label chewable Tablets to indicate that they are to be chewed before swallowing.
- **USP REFERENCE STANDARDS (11)**
 - USP Loperamide Hydrochloride RS
 - USP Loperamide Related Compound F RS
 - Loperamide *trans*-N-oxide; (1*R*,4*S*)-4-(4-Chlorophenyl)-1-[4-(dimethylamino)-4-oxo-3,3-diphenylbutyl]-4-hydroxypiperidine 1-oxide. $C_{29}H_{33}ClN_2O_3$ 493.04

Lopinavir



$C_{37}H_{48}N_4O_5$ 628.80
 [1*S*-(1*R**(*R**),3*R**,4*R**)]-N-[4[(2,6-Dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]-tetrahydro-α-(1-methylethyl)-2-oxo-1(2*H*)-pyrimidineacetamide;
 (α*S*)-Tetrahydro-N-[(α*S*)-α-[(2*S*,3*S*)-2-hydroxy-4-phenyl-3-[2-(2,6-xylyloxy)acetamido]butyl]phenethyl]-α-isopropyl-2-oxo-1(2*H*)-pyrimidineacetamide [192725-17-0].

DEFINITION

Lopinavir contains NLT 98.0% and NMT 102.0% of lopinavir ($C_{37}H_{48}N_4O_5$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197A)**
- **B.** The retention time of the lopinavir peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Buffer: 2.7 g/L of monobasic potassium phosphate and 0.9 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.0. Pass the solution through a suitable filter of 0.45- μ m pore size.

Diluent: Acetonitrile and water (1:1)

Solution A: Acetonitrile and *Buffer* (9:11)

Mobile phase: *Solution A*

Standard solution: 0.025 mg/mL of USP Lopinavir RS in *Diluent*

Sample solution: 0.025 mg/mL of Lopinavir in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm \times 25-cm; 4- μ m packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: 60 min

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 8000 theoretical plates

Capacity factor: NLT 15

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lopinavir ($C_{37}H_{48}N_4O_5$) in the portion of Lopinavir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lopinavir RS in the *Standard solution* (mg/mL)

C_U = concentration of Lopinavir in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.2%

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 μ g/g (Official 1, Jan-2018)

• **ORGANIC IMPURITIES: PROCEDURE 1**

[NOTE—For early-eluting impurities.]

Buffer, Diluent, and Solution A: Prepare as directed in the *Assay*.

Solution B: Acetonitrile and *Buffer* (3:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
60	100	0
61	0	100
81	0	100
82	100	0
100	100	0

System suitability solution: 0.5 mg/mL of USP

Lopinavir System Suitability Mixture RS in *Diluent*

Standard solution: 0.005 mg/mL of USP Lopinavir RS in *Diluent*

Sample solution: 0.5 mg/mL of Lopinavir in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm \times 25-cm; 4- μ m packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: 100 min

[NOTE—Data collection is only for the first 60 min. The remaining gradient steps wash out the late-eluting impurities and re-equilibrate the column.]

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are listed in *Table 2*.]

Suitability requirements

Resolution: NLT 1.2 between lopinavir *N*-formylphenoxacetamide and lopinavir *N*-acetylphenoxacetamide, *System suitability solution*

Capacity factor: NLT 15, *Standard solution*

Column efficiency: NLT 8000, *Standard solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Diluent*, *System suitability solution*, *Standard solution*, and *Sample solution*

Calculate the percentage of each lopinavir related impurity and unidentified impurity in the portion of Lopinavir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of lopinavir from the *Standard solution*

C_S = concentration of USP Lopinavir RS in the *Standard solution* (mg/mL)

C_U = concentration of Lopinavir in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Table 2

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Lopinavir free amine ^a	0.03	0.61	0.1
Lopinavir <i>N</i> -formylaminoalcohol ^b	0.07	0.80	0.2
Lopinavir divalinate ^c	0.10	0.65	0.1
Sulfolopinavir ^d	0.13	0.76	0.1
Lopinavir phenoxyacetamide ^e	0.25	0.96	0.1
Lopinavir <i>N</i> -formylphenoxyacetamide ^f	0.59	1.3	0.1
Lopinavir <i>N</i> -acetylphenoxyacetamide ^g	0.62	1.2	0.1
Lopinavir oxazine ^h	0.90	1.1	0.1
Lopinavir	1.00	—	—
Isolopinavir ⁱ	1.10	0.99	0.2
Lopinavir 2,4-phenoxy isomer ^j	1.13	0.97	0.1
Lopinavir <i>D</i> -valine diastereomer ^k	1.25	1.1	0.1
<i>Z</i> -Diacylthenediamine ^l	1.28	1.4	0.1
Lopinavir (2 <i>R</i> ,4 <i>R</i>) diastereomer ^m	1.32	1.0	0.1
Lopinavir (4 <i>R</i>) epimer ⁿ	1.38	0.97	0.1
Any other individual impurity	—	1.0	0.1

^a (S)-*N*-[(2*S*,4*S*,5*S*)-5-Amino-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

^b (S)-*N*-[(2*S*,4*S*,5*S*)-5-Formamido-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

^c (2*S*,2'*S*)-*N,N'*-[(2*S*,3*S*,5*S*)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis[3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide].

^d (2*S*,3*S*,5*S*)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-[(S)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamido]-1,6-diphenylhexan-3-yl hydrogen sulfate.

^e *N*-[(2*S*,3*S*,5*S*)-5-Amino-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.

^f 2-(2,6-Dimethylphenoxy)-*N*-[(2*S*,3*S*,5*S*)-5-formamido-3-hydroxy-1,6-diphenylhexan-2-yl]acetamide.

^g *N*-[(2*S*,3*S*,5*S*)-5-Acetamido-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.

^h *N*-[(S)-1-[(4*S*,6*S*)-4-Benzyl-2-oxo-1,3-oxazinan-6-yl]-2-phenylethyl]-2-(2,6-dimethylphenoxy)acetamide.

ⁱ (S)-*N*-[(2*S*,3*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-3-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

^j (S)-*N*-[(2*S*,4*S*,5*S*)-5-[2-(2,4-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

^k (R)-*N*-[(2*S*,4*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

^l (Z)-*N,N'*-(Ethene-1,2-diyl)bis[2-(2,6-dimethylphenoxy)acetamide].

^m (S)-*N*-[(2*R*,4*R*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

ⁿ (S)-*N*-[(2*S*,4*R*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

• ORGANIC IMPURITIES: PROCEDURE 2

[NOTE—For late-eluting impurities.]

Buffer, Diluent, and Solution A: Prepare as directed in the Assay.

Solution B: Acetonitrile and Buffer (3:1)

Mobile phase: Solution A and Solution B (3:7)

System suitability solution: 0.5 mg/mL of USP

Lopinavir System Suitability Mixture RS in Diluent

Standard solution: 0.005 mg/mL of USP Lopinavir RS in Diluent

Sample solution: 0.5 mg/mL in Diluent

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 4-μm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: 50 min

System suitability

Sample: Standard solution

[NOTE—The relative retention times are listed in Table 3.]

Suitability requirements

Capacity factor: NLT 1.5

Column efficiency: NLT 3000

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: Diluent, System suitability solution, Standard solution, and Sample solution

Calculate the percentage of each lopinavir related impurity and unidentified impurity in the portion of Lopinavir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the Sample solution

r_S = peak response of lopinavir from the Standard solution

C_S = concentration of USP Lopinavir RS in the Standard solution (mg/mL)

C_U = concentration of Lopinavir in the Sample solution (mg/mL)

F = relative response factor (see Table 3)

Table 3

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Lopinavir	1.00	—	—
Lopinavir <i>O</i> -acyl ^a	1.49	0.77	0.1
Lopinavir (2 <i>R</i>) epimer ^b	1.91	1.1	0.1
Lopinavir diamide ^c	4.39	1.4	0.1
Lopinavir <i>N</i> -acyl ^d	6.01	1.3	0.1
Lopinavir <i>O</i> -phenox-yactyl ^e	7.14	1.1	0.1
Lopinavir aminoalcohol urea ^f	8.46	1.3	0.1

^a (S)-[(2*S*,3*S*,5*S*)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-[(S)-3-methyl-2-(2-oxotetrahydropyrimidin-1(2*H*)-yl)butanamido]-1,6-diphenylhexan-3-yl] 3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoate.

^b (S)-*N*-[(2*R*,4*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

^c *N,N'*-[(2*S*,3*S*,5*S*)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis[2-(2,6-dimethylphenoxy)acetamide].

^d (S)-*N*-[(2*S*,4*S*,5*S*)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-2-[3-[2-(2,6-dimethylphenoxy)acetyl]-2-oxotetrahydropyrimidin-1(2*H*)-yl]-3-methylbutanamide.

^e (2*S*,3*S*,5*S*)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-[(S)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamido]-1,6-diphenylhexan-3-yl 2-(2,6-dimethylphenoxy)acetate.

^f *N,N'*-(2*S*,2'*S*,3*S*,3'*S*,5*S*,5'*S*)-5,5'-Carbonylbis(azanediyl)bis[3-hydroxy-1,6-diphenylhexane-5,2-diyl]bis[2-(2,6-dimethylphenoxy)acetamide].

^g Exclude from Organic Impurities, Procedure 2, lopinavir (4*R*) epimer and any other peak eluting prior to this peak because these are already monitored in Procedure 1.

Table 3 (Continued)

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other individual impurity	—	1.0	0.1
Total impurities from Procedure 1 and Procedure 2	—	1.0	0.7 ^a

^a (S)-[(2S,3S,5S)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-[(S)-3-methyl-2-(2-oxotetrahydropyrimidin-1(2H)-yl)butanamido]-1,6-diphenylhexan-3-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanoate.

^b (S)-N-[(2R,4S,5S)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^c N,N'-[(2S,3S,5S)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis[2-(2,6-dimethylphenoxy)acetamide].

^d (S)-N-[(2S,4S,5S)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-2-[3-(2-(2,6-dimethylphenoxy)acetyl)-2-oxotetrahydropyrimidin-1(2H)-yl]-3-methylbutanamide.

^e (2S,3S,5S)-2-[2-(2,6-Dimethylphenoxy)acetamido]-5-[(S)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamido]-1,6-diphenylhexan-3-yl 2-(2,6-dimethylphenoxy)acetate.

^f N,N'-[(2S,2'S,3S,3'S,5S,5'S)-5,5'-Carbonylbis(azanediyl)]bis[3-hydroxy-1,6-diphenylhexane-5,2-diyl]bis[2-(2,6-dimethylphenoxy)acetamide].

^g Exclude from Organic Impurities, Procedure 2, lopinavir (4R) epimer and any other peak eluting prior to this peak because these are already monitored in Procedure 1.

Acceptance criteria: See Table 2 and Table 3.

SPECIFIC TESTS

- **WATER DETERMINATION**, Method I (921): NMT 4.4%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers. Store at room temperature.
- **USP REFERENCE STANDARDS (11)**
 - USP Lopinavir RS
 - USP Lopinavir System Suitability Mixture RS
 - Lopinavir System Suitability Mixture contains lopinavir N-formylphenoxyacetamide, lopinavir N-acetylphenoxyacetamide, and several other minor components. Lopinavir N-formylphenoxyacetamide is (2-(2,6-dimethylphenoxy)-N-[(2S,3S,5S)-5-formamido-3-hydroxy-1,6-diphenylhexan-2-yl]acetamide. $C_{29}H_{34}N_2O_4$ 474.59
 - Lopinavir N-acetylphenoxyacetamide is (N-[(2S,3S,5S)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide. $C_{30}H_{36}N_2O_4$ 488.62

Lopinavir and Ritonavir Oral Solution

DEFINITION

Lopinavir and Ritonavir Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$).

IDENTIFICATION

- **A.** The retention times of the lopinavir and ritonavir peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

ASSAY

• LOPINAVIR AND RITONAVIR

Buffer: 4.1 g/L of monobasic potassium phosphate in water

Solution A: Acetonitrile and *Buffer* (65:35)

Solution B: Acetonitrile and *Buffer* (50:50)

Mobile phase: Acetonitrile, methanol, tetrahydrofuran, and *Buffer* (175:100:100:625). Separately filter the *Buffer* and the premixed solvents before combining them to make the *Mobile phase*.

Standard stock solution: 0.1 mg/mL each of USP

Lopinavir RS and USP Ritonavir RS in *Solution B*

Standard solution: 0.025 mg/mL each of USP Lopinavir RS and USP Ritonavir RS in *Solution B* from the *Standard stock solution*

Sample stock solution: Nominally 4 mg/mL of lopinavir and 1 mg/mL of ritonavir in *Solution A* prepared as follows. Transfer a volume of Oral Solution equivalent to 400 mg of lopinavir and 100 mg of ritonavir to a 100-mL volumetric flask with the aid of several small portions of *Solution A*, and then dilute with *Solution A* to volume.

Sample solution: Nominally 0.05 mg/mL of lopinavir and 0.0125 mg/mL of ritonavir in *Solution B* from the *Sample stock solution*

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.2 for the ritonavir peak

Relative standard deviation: NMT 2.0% each for ritonavir and lopinavir

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*

r_S = peak response of the corresponding analyte from the *Standard solution*

C_S = concentration of USP Lopinavir RS or USP Ritonavir RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lopinavir or ritonavir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$)

PERFORMANCE TESTS• **DELIVERABLE VOLUME** (698)

For multiple-unit containers

Acceptance criteria: Meets the requirements

IMPURITIES• **ORGANIC IMPURITIES****Buffer A:** 4.1 g/L of monobasic potassium phosphate in water**Buffer B:** 3.8 g/L of monobasic potassium phosphate and 0.25 g/L of dibasic potassium phosphate in water**Solution A:** Acetonitrile and *Buffer A* (50:50)**Solution B:** Acetonitrile, butyl alcohol, and *Buffer A* (15:5:80)**Solution C:** Acetonitrile and *Buffer A* (65:35)**Mobile phase:** Acetonitrile, tetrahydrofuran, butyl alcohol, and *Buffer B* (18:8:5:69). Adjust with 1 M phosphoric acid or 1 M potassium hydroxide, if necessary, to a pH of 6.3.**Standard stock solution:** 0.1 mg/mL each of USP Lopinavir RS and USP Ritonavir RS in *Solution A***Standard solution:** 0.01 mg/mL each of USP Lopinavir RS and USP Ritonavir RS from *Standard stock solution* in *Solution B***Peak identification solution:** Transfer a weighed portion of Oral Solution to a crimp-top container. Add an amount of citric acid equivalent to 1% by weight of the Oral Solution taken and mix until dissolved. Seal the container, and heat at 50° for approximately 4 days. Use this degradation solution and follow the procedure described below in the *Sample stock solution* and *Sample solution* sections to prepare the *Peak identification solution*.**Sample stock solution:** Transfer 5 mL of Oral Solution with the aid of several small portions of *Solution C* to a 100-mL volumetric flask, and dilute with *Solution C* to volume.**Sample solution:** Dilute 25.0 mL of *Sample stock solution* with *Solution B* to 50.0 mL. Transfer 15.0 mL of this solution to a 50-mL centrifuge tube that has been previously rinsed with methanol and dried. Add 20.0 mL of *n*-heptane, and shake vigorously until a uniform emulsion is formed. Vent the tube periodically while shaking. Centrifuge the emulsion for about 5 min. Carefully remove the top heptane layer by aspiration, leaving the clear *Sample solution* layer. The middle viscous, cloudy layer should be considered part of the heptane layer for removal by aspiration. Precondition a strong anion-exchange cartridge (quaternary ammonium functionality on a styrene/divinylbenzene base) with a sorbent mass of 600 mg by rinsing the cartridge with 3 mL of methanol, then 3 mL of *Solution C*, and repeating these rinse steps. Dry the cartridge for about 10 min with the aid of a low vacuum. Transfer 5.0 mL of the clear *Sample*solution to the preconditioned cartridge. With the aid of a vacuum, slowly pass the *Sample solution* completely through the cartridge, collect the extract in a 5-mL volumetric flask, and then dilute with *Solution C* to volume.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 215 nm and 240 nm**Column:** 4.6-mm × 15-cm; 3-μm packing L26**Column temperature:** 60°**Flow rate:** 1 mL/min**Injection volume:** 50 μL**Run time:** 2 times the retention time of lopinavir**System suitability****Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 2.5 between the ritonavir and lopinavir peaks at 215 nm**Tailing factor:** 0.8–1.2 for the ritonavir peak at 240 nm**Relative standard deviation:** NMT 3.0% for the lopinavir peak at 215 nm; NMT 3.0% for the ritonavir peak at 240 nm**Analysis****Samples:** *Standard solution*, *Peak identification solution*, and *Sample solution*[NOTE—Determine the relative retention values (*r*) for the components listed in *Table 1* and *Table 2*, using the time measured at the first baseline deflection of the *Standard solution* chromatogram as the void volume (*t_M*).]To identify the ritonavir impurities, determine the relative retention value from the 240-nm chromatogram relative to ritonavir (see *Table 1*). The *Peak identification solution* may also be used to identify ritonavir degradants. Unspecified ritonavir impurities are assigned according to the algorithm outlined in *Table 3*. Calculate the percentage of each ritonavir impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each individual impurity from the *Sample solution**r_s* = peak response of ritonavir from the *Standard solution**C_s* = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)*C_u* = nominal concentration of ritonavir in the *Sample solution* (mg/mL)*F* = relative response factor (see *Table 1*)Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Value (r)	Relative Response Factor	Acceptance Criteria, NMT (%)
Ureidovaline ^a	0.03	—	— ^b
N-Deacylvaline ritonavir ^c	0.11	0.81	0.8
Glycerol carbamate analog ^d	0.14	0.62	0.2
Acetamidoalcohol ^e	0.15	—	— ^b
Hydroxypropyl carbamate analog ^f	0.24 ^g	0.59	0.5
2,5-Thiazolylmethylidicarbamate ^h	0.24 ⁱ	—	— ^b
Hydroxyritonavir ^j	0.36	0.86	0.3
Hydantoin aminoalcohol ^k	0.39	0.73	0.4
Ritonavir hydroperoxide ^l	0.44 ^j	0.88	0.2
Ethyl carbamate analog ^m	0.45 ^j	0.66	0.7
Hydantoin-oxazolidinone derivative ⁿ	0.50	—	— ^b
Ethyl analog ^o	0.64	—	— ^b
O-Acyl isomer ^p	0.74	1.1	0.2
BOC-aminoalcohol ^q	0.81	—	— ^b
Isobutoxycarbonyl aminoalcohol ^r	0.81	—	— ^b
Oxazolidinone derivative ^s	0.87	0.53	0.2
Ureidovaline isobutyl ester ^t	0.94	—	— ^b
Ritonavir	1.0	—	—
4-Hydroxy isomer ^u	1.05	—	— ^b
3R-Epimer ^v	1.11	—	— ^b
Aminoalcohol urea derivative ^w	1.14	—	— ^b
3R,5R-Epimer ^x	1.23	—	— ^b
5R-Epimer ^y	1.32	—	— ^b
Diacyl valine urea ^z	1.70	—	— ^b
Any unspecified ritonavir impurity	—	1.0	0.2
Total ritonavir impurities, specified and unspecified	—	—	3.0 ^{aa}

^a [N-Methyl[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl-L-valine.

^b These are process impurities which are included in this table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and are not included in the total impurities.

^c Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^d Thiazol-5-ylmethyl (2S,3S,5S)-5-[(2S)-2-(2,3-dihydroxypropoxycarbonylamino)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^e Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-ethoxycarbonylamino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^f Thiazol-5-ylmethyl (2S,3S,5S)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^g If two peaks appear with a relative retention value of 0.24, the second peak will be identified as 2,5-thiazolylmethylidicarbamate.

^h 2,5-Thiazolylmethylidicarbamate.

ⁱ A single peak with a relative retention value of 0.44 should be reported as the ethyl carbamate analog due to possible coelution with ritonavir hydroperoxide impurity.

^j Thiazol-5-ylmethyl (2S,3S,5S)-3-hydroxy-5-[[2-(2-hydroxypropan-2-yl)thiazol-4-yl]methyl]-3-methylureido]acetamido]-1,6-diphenylhexan-2-ylcarbamate.

^k Thiazol-5-ylmethyl (2S,3S,5S)-3-hydroxy-5-[(S)-4-isopropyl-2,5-dioximidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.

^l Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-ethoxycarbonylamino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^m Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-{3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

ⁿ (4S,5S)-Thiazol-5-ylmethyl 4-benzyl-5-[(S)-2-[(S)-4-isopropyl-2,5-dioximidazolidin-1-yl]-3-phenylpropyl]-2-oxooxazolidine-3-carboxylate.

^o Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-{3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^p (S)-[(2S,3S,5S)-5-Amino-1,6-diphenyl-2-[(thiazol-5-ylmethoxy)carbonylamino]hexan-3-yl] 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.

^q Thiazol-5-ylmethyl (2S,3S,5S)-5-(t-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^r Thiazol-5-ylmethyl (2S,3S,5S)-5-(isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^s (S)-N-[(S)-1-[(4S,5S)-4-Benzyl-2-oxooxazolidin-5-yl]-3-phenylpropan-2-yl]-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamide.

^t (S)-Isobutyl 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.

^u Thiazol-5-ylmethyl (2S,4S,5S)-4-hydroxy-5-[(S)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^v Thiazol-5-ylmethyl (2S,3R,5S)-3-hydroxy-5-[(S)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^w Bis(thiazol-5-ylmethyl) (2S,2'S,3S,3'S,5S,5'S)-5,5'-carbonylbis(azanediyl)bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)dicarbamate.

^x Thiazol-5-ylmethyl (2S,3R,5R)-3-hydroxy-5-[(S)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^y Thiazol-5-ylmethyl (2S,3S,5R)-3-hydroxy-5-[(S)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^z (S)-N-[(2S,4S,5S)-5-(Thiazol-5-ylmethoxycarbonylamino)-4-hydroxy-1,6-diphenylhexan-2-yl]-2-{3-[(2S,4S,5S)-5-(thiazol-5-ylmethoxycarbonylamino)-4-hydroxy-1,6-diphenylhexan-2-yl]ureido}-3-methylbutanamide.

^{aa} Disregard any peak less than 0.01% in the calculation of total impurities.

To identify the lopinavir impurities, determine the relative retention value from the 215-nm chromatogram relative to ritonavir (see Table 2). Compare the 215-nm chromatogram to the 240-nm chromatogram.

Any impurity assigned as a ritonavir impurity at 240 nm that is also observed at 215 nm is discounted. Unspecified ritonavir impurities are assigned according to the algorithm outlined in Table 3.

Calculate the percentage of each unspecified lopinavir impurity at 215 nm in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of lopinavir from the *Standard solution*

C_S = concentration of USP Lopinavir RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lopinavir in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Value (r)	Acceptance Criteria, NMT (%)
Lopinavir aminoalcohol ^a	0.06	— ^b
Lopinavir <i>N</i> -formylaminoalcohol ^c	0.12	— ^b
Lopinavir divalinate ^d	0.21	— ^b
Lopinavir phenoxyacetamide ^e	0.35	— ^b
Lopinavir <i>N</i> -formylphenoxyacetamide ^f	0.67	— ^b
Lopinavir <i>N</i> -acetylphenoxyacetamide ^g	0.69	— ^b
Lopinavir oxazine ^h	0.77	— ^b
<i>Z</i> -Diacylthenediamine ⁱ	0.92	— ^b
Ritonavir	1.0	— ^b
Isolopinavir	1.18	— ^b
Lopinavir 2,4-dimethylphenoxy isomer ^k	1.21	— ^b
Lopinavir 4-epimer ^l	1.26	— ^b

^a (S)-N-[(2S,4S,5S)-5-Amino-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^b These are process impurities which are included in this table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and are not included in the total impurities.

^c (S)-N-[(2S,4S,5S)-5-Formamido-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^d (2S,2'S)-N,N'-[(2S,3S,5S)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis[3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide].

^e N-[(2S,3S,5S)-5-Amino-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.

^f 2-(2,6-Dimethylphenoxy)-N-[(2S,3S,5S)-5-formamido-3-hydroxy-1,6-diphenylhexan-2-yl]acetamide.

^g N-[(2S,3S,5S)-5-Acetamido-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.

^h N-[(S)-1-[(4S,6S)-4-Benzyl-2-oxo-1,3-oxazinan-6-yl]-2-phenylethyl]-2-(2,6-dimethylphenoxy)acetamide.

ⁱ (Z)-N,N'-(Ethene-1,2-diyl)bis[2-(2,6-dimethylphenoxy)acetamide].

^j (S)-N-[(2S,3S,5S)-5-[2-(2,6-Dimethylphenoxy)acetamido]-3-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^k (S)-N-[(2S,4S,5S)-5-[2-(2,4-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^l (S)-N-[(2S,4R,5S)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^m (R)-N-[(2S,4S,5S)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

ⁿ (S)-N-[(2R,4R,5S)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^o (S)-N-[(2R,4S,5S)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^p Disregard any peak less than 0.01%.

Table 2 (Continued)

Name	Relative Retention Value (r)	Acceptance Criteria, NMT (%)
Lopinavir D-valine diastereomer ^m	1.33	— ^b
Lopinavir (2R,4R) diastereomer ⁿ	1.42	— ^b
Lopinavir 2-epimer ^o	1.79	— ^b
Any unspecified lopinavir impurity	—	0.2 ^p
Total unspecified lopinavir impurities	—	0.5 ^p

^a (S)-N-[(2S,4S,5S)-5-Amino-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^b These are process impurities which are included in this table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and are not included in the total impurities.

^c (S)-N-[(2S,4S,5S)-5-Formamido-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^d (2S,2'S)-N,N'-[(2S,3S,5S)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis[3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide].

^e N-[(2S,3S,5S)-5-Amino-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.

^f 2-(2,6-Dimethylphenoxy)-N-[(2S,3S,5S)-5-formamido-3-hydroxy-1,6-diphenylhexan-2-yl]acetamide.

^g N-[(2S,3S,5S)-5-Acetamido-3-hydroxy-1,6-diphenylhexan-2-yl]-2-(2,6-dimethylphenoxy)acetamide.

^h N-[(S)-1-[(4S,6S)-4-Benzyl-2-oxo-1,3-oxazinan-6-yl]-2-phenylethyl]-2-(2,6-dimethylphenoxy)acetamide.

ⁱ (Z)-N,N'-(Ethene-1,2-diyl)bis[2-(2,6-dimethylphenoxy)acetamide].

^j (S)-N-[(2S,3S,5S)-5-[2-(2,6-Dimethylphenoxy)acetamido]-3-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^k (S)-N-[(2S,4S,5S)-5-[2-(2,4-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^l (S)-N-[(2S,4R,5S)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^m (R)-N-[(2S,4S,5S)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

ⁿ (S)-N-[(2R,4R,5S)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^o (S)-N-[(2R,4S,5S)-5-[2-(2,6-Dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl]butanamide.

^p Disregard any peak less than 0.01%.

For calculating and reporting impurities, follow the algorithm outlined in Table 3.

Table 3

Wavelength (nm)	Unspecified Impurity Observed	
240	Yes	No
215	Yes or No	Yes
Peak assigned to	Ritonavir	Lopinavir
Quantitation wavelength	240 nm	215 nm

SPECIFIC TESTS

• ALCOHOL DETERMINATION (611)

Internal standard solution: Transfer 10.0 mL of butyl alcohol to a 200-mL volumetric flask and dilute with methanol to volume.

Internal standard identification solution: Dilute 5.0 mL of *Internal standard solution* with methanol to 100 mL.

Standard stock solution: 4.0% (v/v) of dehydrated alcohol in methanol

Standard solution: 0.4% (v/v) of dehydrated alcohol prepared as follows. Transfer 10.0 mL of *Standard stock*

solution and 5.0 mL of the *Internal standard solution* to a 100-mL volumetric flask, and dilute with methanol to volume.

Sample stock solution: Transfer 5.0 mL of Oral Solution to a 50-mL volumetric flask with the aid of several portions of methanol, and dilute with methanol to volume.

Sample solution: Transfer 10.0 mL of *Sample stock solution* and 5.0 mL of the *Internal standard solution* to a 100-mL volumetric flask, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m fused silica capillary; coated with a 1-μm film of liquid phase G16

Temperatures

Injection port: 185°

Detector: 220°

Column: See *Table 4*.

Table 4

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	5
40	10	145	6
145	20	200	9.75

Carrier gas: Helium

Flow rate: 4.5 mL/min

Makeup gas flow: 30 mL/min

Injection volume: 1 μL

Injection type: Split ratio 4:1

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.2 for the alcohol peak

Relative standard deviation: NMT 3.0% for the peak area ratio of alcohol to the internal standard

Analysis

Samples: *Internal standard identification solution*, *Standard solution*, and *Sample solution*

Calculate the percentage of the labeled amount of alcohol in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times D \times 100$$

R_U = peak response ratio of alcohol to butyl alcohol from the *Sample solution*

R_S = peak response ratio of alcohol to butyl alcohol from the *Standard solution*

C_S = concentration of dehydrated alcohol in the *Standard solution* (% v/v)

C_U = nominal concentration of alcohol in the Oral Solution (% v/v)

D = dilution factor used to prepare the *Sample solution*

Acceptance criteria: 85.0%–115.0% of the labeled amount of alcohol (C_2H_6O)

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed 10^2 cfu/mL.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at 2°–8°.

• USP REFERENCE STANDARDS (11)

USP Lopinavir RS

USP Ritonavir RS

Lopinavir and Ritonavir Tablets

DEFINITION

Lopinavir and Ritonavir Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

ASSAY

• LOPINAVIR AND RITONAVIR

Buffer 1: 4.1 g/L of monobasic potassium phosphate in water

Solution A: Acetonitrile and Buffer 1 (50:50)

Buffer 2: 2.1 g/L of monobasic potassium phosphate in water

Solution B: Acetonitrile and 1-butanol (13:3)

Solution C: Acetonitrile, 1-butanol, Buffer 1, and water (65:15:10:10)

Standard solution: 6.25 μg/mL of USP Ritonavir RS and 25 μg/mL of USP Lopinavir RS in *Solution A*

Sample solution: Place a number of Tablets equivalent to 1000 mg of lopinavir and 250 mg of ritonavir in a 250-mL volumetric flask, add 25 mL of Buffer 2, and agitate to dissolve the Tablet coating, if necessary. Add 100 mL of *Solution B*, and shake mechanically until the Tablets are dissolved. Dilute with *Solution C* to volume. Centrifuge a portion of this solution, and then further dilute with *Solution A* to a nominal concentration of 6.25 μg/mL of ritonavir and 25 μg/mL of lopinavir.

Mobile phase: Acetonitrile, methanol, tetrahydrofuran, and Buffer 1 (175:100:100:625)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 μL

System suitability

Sample: *Standard solution*

[NOTE—The elution order is ritonavir, then lopinavir.]

Suitability requirements

Capacity factor: 15–24 for the ritonavir peak

Tailing factor: 0.8–1.2 for the ritonavir peak

Theoretical plates: More than 5000 for the ritonavir peak

Relative standard deviation: NMT 2.0% for the ritonavir and lopinavir peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lopinavir ($C_{37}H_{48}N_4O_5$) and ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lopinavir or ritonavir from the *Sample solution*

r_S = peak response of lopinavir or ritonavir from the *Standard solution*

C_S = concentration of lopinavir or ritonavir in the *Standard solution* (μg/mL)

C_U = nominal concentration of lopinavir or ritonavir in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0% of the labeled amounts of lopinavir ($\text{C}_{37}\text{H}_{48}\text{N}_4\text{O}_5$) and ritonavir ($\text{C}_{37}\text{H}_{48}\text{N}_6\text{O}_5\text{S}_2$)

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 60 mM polyoxyethylene 10 lauryl ether (37.56 g/L) in water; 900 mL

Apparatus 2: 75 rpm

Time: 90 min

Mobile phase: Acetonitrile and 4.1 g/L potassium phosphate monobasic (55:45). Adjust with phosphoric acid to an apparent pH of 4.0 ± 0.05 .

Standard solution: Dissolve USP Lopinavir RS in methanol to obtain a solution containing 2.6 mg/mL. Dissolve USP Ritonavir RS in methanol to obtain a solution containing 1.3 mg/mL. Combine portions of these solutions to make a solution containing approximately 0.104 mg/mL of lopinavir and 0.026 mg/mL of ritonavir in *Medium*.

Sample solutions: Pass a portion of the solution under test through a suitable filter. If necessary, dilute the solution with *Medium* to obtain a final sample solution containing approximately 0.104 mg/mL of lopinavir and 0.026 mg/mL of ritonavir.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm \times 15-cm; 5- μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 25 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between lopinavir and ritonavir peaks

Relative standard deviation: NMT 2.0% for the lopinavir and ritonavir peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lopinavir ($\text{C}_{37}\text{H}_{48}\text{N}_4\text{O}_5$) and ritonavir ($\text{C}_{37}\text{H}_{48}\text{N}_6\text{O}_5\text{S}_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

r_U = peak response of lopinavir or ritonavir from the *Sample solution*

r_S = peak response of lopinavir or ritonavir from the *Standard solution*

C_S = concentration of USP Lopinavir RS or USP Ritonavir RS in the *Standard solution* (mg/mL)

L = Tablet label claim for lopinavir or ritonavir (mg)

D = dilution factor of the *Sample solution*

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amounts of lopinavir ($\text{C}_{37}\text{H}_{48}\text{N}_4\text{O}_5$) and ritonavir ($\text{C}_{37}\text{H}_{48}\text{N}_6\text{O}_5\text{S}_2$) are dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Buffer 1: 4.1 g/L of monobasic potassium phosphate in water

Solution A: *Buffer 1* and acetonitrile (50:50)

Buffer 2: 2.1 g/L of monobasic potassium phosphate in water

Solution B: Acetonitrile, 1-butanol, and *Buffer 1* (15:5:80)

Solution C: Acetonitrile, 1-butanol, *Buffer 1*, and water (65:15:10:10)

Solution D: Acetonitrile and 1-butanol (13:3)

Buffer solution: 3.8 g/L of monobasic potassium phosphate and 0.25 g/L of dibasic potassium phosphate in water

Mobile phase: Acetonitrile, tetrahydrofuran, 1-butanol, and *Buffer solution* (18:8:5:69). Adjust with 1 M phosphoric acid or 1 M potassium hydroxide, if necessary, to a pH of 6.3 ± 0.1 .

Standard stock solution: 0.025 mg/mL of USP Ritonavir RS in *Solution A*

Standard solution: 2.5 $\mu\text{g/mL}$ of USP Ritonavir RS in *Solution B* from *Standard stock solution*

Ritonavir degradant identification solution: Transfer two 5.0 mL portions of a 1 mg/mL solution of USP Ritonavir RS in *Solution A* to separate 50-mL volumetric flasks. Add 1 g of citric acid to one flask, and shake until dissolved. Heat both flasks at 80° for approximately 24 h. Cool the flasks, and add 13 mL of 1 N sodium hydroxide to the flask containing the citric acid. Dilute both flasks with *Solution B* to volume. Combine equal volumes of both solutions. This solution contains ritonavir and the ritonavir degradation products (*N*-deacylvaline ritonavir, hydantoin aminoalcohol, *O*-acyl isomer, and oxazolidinone derivative).

Ritonavir related compounds identification solution: 1 mg/mL of USP Ritonavir Related Compounds Mixture RS dissolved in *Solution C* and further diluted with *Solution B* to 0.5 mg/mL.

Sample solution: Place a number of Tablets equivalent to 1000 mg of lopinavir and 250 mg of ritonavir into a 250-mL volumetric flask. Add 25 mL of *Buffer 2*, and agitate to dissolve the Tablet coating, if necessary. Add 100 mL of *Solution D*, and shake mechanically until the Tablets are dissolved. Dilute with *Solution C* to volume. Centrifuge a portion of this solution, and further dilute with *Solution B* to a concentration of 2 mg/mL of lopinavir and 0.5 mg/mL of ritonavir.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Column: 4.6-mm \times 15-cm; 3- μm packing L26

Column temperature: 60°

Detector: UV 240 nm

Injection volume: 50 μL

Flow rate: 1.0 mL/min

System suitability

Samples: *Ritonavir degradant identification solution*, *Ritonavir related compounds identification solution*, and *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between the peaks for *O*-acyl isomer and oxazolidinone derivative, *Ritonavir degradant identification solution*. NLT 0.7 between the peaks for hydroxyritonavir and hydantoin aminoalcohol, *Ritonavir related compounds identification solution*

Capacity factor: NLT 10.8, *Standard solution*

Tailing factor: 0.8–1.2, *Standard solution*

Column efficiency: NLT 5000, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each ritonavir degradation product in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of individual degradation product from the *Sample solution*

r_S = peak response of ritonavir from the *Standard solution*

C_S = concentration of USP Ritonavir RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ritonavir in the Sample solution (mg/mL)

F = relative response factor

Acceptance criteria: See Table 1. [NOTE—Disregard all peaks eluting before the retention time of the *N*-deacylvaline ritonavir peak from the Ritonavir degradant identification solution.]

Table 1

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
<i>N</i> -Deacylvaline ritonavir ^a	0.11	0.81	0.2
Acetamidolcohol ^b	0.15	—	—*
2,5-Thiazolylmethyl-dicarbamate ^c	0.24	—	—*
Hydroxyritonavir ^d	0.36	0.86	0.3
Hydantoin aminoalcohol ^e	0.39	0.73	2.6
Ritonavir hydroperoxide ^f	0.44	0.88	0.2
Hydantoin-oxazolidinone derivative ^g	0.50	—	—*

^a Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^b Thiazol-5-ylmethyl (2S,3S,5S)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^c Bis(thiazol-5-ylmethyl) (2S,3S,5S)-3-hydroxy-1,6-diphenylhexane-2,5-diyl dicarbamate.

^d Thiazol-5-ylmethyl (2S,3S,5S)-3-hydroxy-5-[(S)-2-(3-[(2-hydroxypropan-2-yl)thiazol-4-yl]methyl)-3-methylureido]-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^e Thiazol-5-ylmethyl (2S,3S,5S)-3-hydroxy-5-[(S)-4-isopropyl-2,5-dioximidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.

^f Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-(3-[(2-hydroperoxypropan-2-yl)thiazol-4-yl]methyl)-3-methylureido]-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^g (4S,5S)-Thiazol-5-ylmethyl 4-benzyl-5-[(S)-2-[(S)-4-isopropyl-2,5-dioximidazolidin-1-yl]-3-phenylpropyl]-2-oxooxazolidine-3-carboxylate.

^h Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-(3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

ⁱ (S)-[(2S,3S,5S)-5-Amino-1,6-diphenyl-2-[(thiazol-5-ylmethoxy)carbonylamino]hexan-3-yl] 2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanoate.

^j Thiazol-5-ylmethyl (2S,3S,5S)-5-(*t*-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^k Thiazol-5-ylmethyl (2S,3S,5S)-5-(isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^l (S)-N-[(S)-1-[(4S,5S)-4-Benzyl-2-oxooxazolidin-5-yl]-3-phenylpropan-2-yl]-2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanoate.

^m (S)-Isobutyl 2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanoate.

ⁿ Thiazol-5-ylmethyl (2S,4S,5S)-4-hydroxy-5-[(S)-2-(3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^o Thiazol-5-ylmethyl (2S,3R,5S)-3-hydroxy-5-[(S)-2-(3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^p Bis(thiazol-5-ylmethyl) (2S,2'S,3S,3'S,5S,5'S)-5,5'-carbonylbis(azanediyl)bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)dicarbamate.

^q Thiazol-5-ylmethyl (2S,3R,5R)-3-hydroxy-5-[(S)-2-(3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^r Thiazol-5-ylmethyl (2S,3S,5R)-3-hydroxy-5-[(S)-2-(3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^s (3S,4S,6S,10S,13S,15S,16S)-Bis(thiazol-5-ylmethyl)-4,15-dihydroxy-10-isopropyl-8,11-dioxo-3,6,13,16-tetrabenzyl-2,7,9,12,17-pentaazaoctadecanedioate.

* Process impurities; for information only.

** Disregard any peak less than 0.05%.

Table 1 (Continued)

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Ethyl analog ^h	0.64	—	—*
O-Acyl isomer ⁱ	0.74	1.1	0.2
BOC-aminoalcohol ^j	0.81	—	—*
Isobutoxycarbonyl aminoalcohol ^k	0.81	—	—*
Oxazolidinone derivative ^l	0.87	0.53	0.3
Ureidovaline isobutyl ester ^m	0.94	—	—*
Ritonavir	1.0	—	—*
4-Hydroxy isomer ⁿ	1.05	—	—*
3R-Epimer ^o	1.11	—	—*
Aminoalcohol urea derivative ^p	1.14	—	—*
3R,5R-Epimer ^q	1.23	—	—*
5R-Epimer ^r	1.32	—	—*
Diacyl valine urea ^s	1.70	—	—*

^a Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^b Thiazol-5-ylmethyl (2S,3S,5S)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^c Bis(thiazol-5-ylmethyl) (2S,3S,5S)-3-hydroxy-1,6-diphenylhexane-2,5-diyl dicarbamate.

^d Thiazol-5-ylmethyl (2S,3S,5S)-3-hydroxy-5-[(S)-2-(3-[(2-hydroxypropan-2-yl)thiazol-4-yl]methyl)-3-methylureido]-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^e Thiazol-5-ylmethyl (2S,3S,5S)-3-hydroxy-5-[(S)-4-isopropyl-2,5-dioximidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.

^f Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-(3-[(2-hydroperoxypropan-2-yl)thiazol-4-yl]methyl)-3-methylureido]-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^g (4S,5S)-Thiazol-5-ylmethyl 4-benzyl-5-[(S)-2-[(S)-4-isopropyl-2,5-dioximidazolidin-1-yl]-3-phenylpropyl]-2-oxooxazolidine-3-carboxylate.

^h Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-(3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

ⁱ (S)-[(2S,3S,5S)-5-Amino-1,6-diphenyl-2-[(thiazol-5-ylmethoxy)carbonylamino]hexan-3-yl] 2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanoate.

^j Thiazol-5-ylmethyl (2S,3S,5S)-5-(*t*-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^k Thiazol-5-ylmethyl (2S,3S,5S)-5-(isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^l (S)-N-[(S)-1-[(4S,5S)-4-Benzyl-2-oxooxazolidin-5-yl]-3-phenylpropan-2-yl]-2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanoate.

^m (S)-Isobutyl 2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanoate.

ⁿ Thiazol-5-ylmethyl (2S,4S,5S)-4-hydroxy-5-[(S)-2-(3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^o Thiazol-5-ylmethyl (2S,3R,5S)-3-hydroxy-5-[(S)-2-(3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^p Bis(thiazol-5-ylmethyl) (2S,2'S,3S,3'S,5S,5'S)-5,5'-carbonylbis(azanediyl)bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)dicarbamate.

^q Thiazol-5-ylmethyl (2S,3R,5R)-3-hydroxy-5-[(S)-2-(3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^r Thiazol-5-ylmethyl (2S,3S,5R)-3-hydroxy-5-[(S)-2-(3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^s (3S,4S,6S,10S,13S,15S,16S)-Bis(thiazol-5-ylmethyl)-4,15-dihydroxy-10-isopropyl-8,11-dioxo-3,6,13,16-tetrabenzyl-2,7,9,12,17-pentaazaoctadecanedioate.

* Process impurities; for information only.

** Disregard any peak less than 0.05%.

Table 1 (Continued)

Name	Relative Retention	Relative Response Factor	Acceptance Criteria, NMT (%)
Any unspecified impurity	—	1.0	0.2**
Total impurities	—	—	3.5**

^a Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^b Thiazol-5-ylmethyl (2S,3S,5S)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^c Bis(thiazol-5-ylmethyl) (2S,3S,5S)-3-hydroxy-1,6-diphenylhexane-2,5-diyl dicarbamate.

^d Thiazol-5-ylmethyl (2S,3S,5S)-3-hydroxy-5-[(S)-2-(3-[(2-(2-hydroxypropan-2-yl)thiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^e Thiazol-5-ylmethyl (2S,3S,5S)-3-hydroxy-5-[(S)-4-isopropyl-2,5-dioximidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.

^f Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-(3-[(2-(2-hydroxypropan-2-yl)thiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^g (4S,5S)-Thiazol-5-ylmethyl 4-benzyl-5-[(S)-2-(3-[(2-(2-hydroxypropan-2-yl)thiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^h Thiazol-5-ylmethyl (2S,3S,5S)-5-[(S)-2-(3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

ⁱ (S)-[(2S,3S,5S)-5-Amino-1,6-diphenyl-2-[(thiazol-5-ylmethoxy)carbonylamino]hexan-3-yl]-2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanoate.

^j Thiazol-5-ylmethyl (2S,3S,5S)-5-(t-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^k Thiazol-5-ylmethyl (2S,3S,5S)-5-(isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

^l (S)-N-[(S)-1-[(4S,5S)-4-Benzyl-2-oxoimidazolidin-5-yl]-3-phenylpropan-2-yl]-2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanamide.

^m (S)-isobutyl 2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanoate.

ⁿ Thiazol-5-ylmethyl (2S,4S,5S)-4-hydroxy-5-[(S)-2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^o Thiazol-5-ylmethyl (2S,3R,5S)-3-hydroxy-5-[(S)-2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^p Bis(thiazol-5-ylmethyl) (2S,2'S,3S,3'S,5S,5'S)-5,5'-carbonylbis(azanediy)bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)dicarbamate.

^q Thiazol-5-ylmethyl (2S,3R,5R)-3-hydroxy-5-[(S)-2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^r Thiazol-5-ylmethyl (2S,3S,5R)-3-hydroxy-5-[(S)-2-[3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido]-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

^s (3S,4S,6S,10S,13S,15S,16S)-Bis(thiazol-5-ylmethyl)-4,15-dihydroxy-10-isopropyl-8,11-dioxo-3,6,13,16-tetrabenzyl-2,7,9,12,17-pentaazaoctadecanedioate.

^{*} Process impurities; for information only.

^{**} Disregard any peak less than 0.05%.

ADDITIONAL REQUIREMENTS

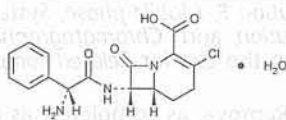
• USP REFERENCE STANDARDS (11)

USP Lopinavir RS

USP Ritonavir RS

USP Ritonavir Related Compounds Mixture RS

Loracarbef



C₁₆H₁₆ClN₃O₄ · H₂O 367.78

1-Azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-chloro-8-oxo-, monohydrate, [6R-[6α,7β(R*)]]- (6R,7S)-7-[(R)-2-Amino-2-phenylacetamido]-3-chloro-8-oxo-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monohydrate [121961-22-6].

Anhydrous 349.78

» Loracarbef contains not less than 960 µg and not more than 1020 µg of anhydrous loracarbef (C₁₆H₁₆ClN₃O₄) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Cefaclor RS

USP Loracarbef RS

USP Loracarbef L-Isomer RS

Identification—

A: Infrared Absorption (197K).

B: The retention time of the loracarbef peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Specific rotation (781S): between +27° and +33°, calculated on the anhydrous basis, determined in a solution in 0.1 N hydrochloric acid containing 10 mg in each mL.

Crystallinity (69S): meets the requirements.

pH (791): between 3.0 and 5.5, in a suspension (1 in 10).

Water Determination, Method I (921): between 3.5% and 6.0%.

Related compounds—

Solution A—Dissolve 6.9 g of monobasic ammonium phosphate in 1960 mL of water. Adjust with phosphoric acid to a pH of 2.5, add 40 mL of acetonitrile, and mix. Filter, if necessary, to obtain a clear solution, and degas.

Solution B—Dissolve 6.9 g of monobasic ammonium phosphate in 600 mL of water. Adjust with phosphoric acid to a pH of 2.5, add 1400 mL of acetonitrile, and mix. Filter, if necessary, to obtain a clear solution, and degas.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*.

NOTE—When preparing the *System suitability solution*, the *Standard solution*, and the *Test solution*, if necessary, sonicate and mix on a vortex mixer to aid in dissolution. Use the solutions immediately after preparation or refrigerate and use within 24 hours.

Phenylglycine solution—Dissolve an accurately weighed quantity of phenylglycine in *Solution A* to obtain a solution having a known concentration of about 0.0075 mg per mL.

System suitability solution—Dissolve accurately weighed quantities of USP Loracarbef RS and USP Cefaclor RS in *Solution A* to obtain a solution having known concentrations of about 0.01 mg of each per mL.

Standard solution—Dissolve an accurately weighed quantity of USP Loracarbef RS in *Solution A* to obtain a solution having a known concentration of about 0.01 mg per mL.

Test solution—Transfer about 50 mg of Loracarbef, accurately weighed, to a 10-mL volumetric flask, add about 8 mL of *Solution A*, and dissolve. Dilute with *Solution A* to volume, and mix. Filter, if necessary, to obtain a clear solution.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 2 mL per minute, and is maintained at a constant temperature of about 40°. The chromatograph is programmed as follows. Initially it is equilibrated with *Solution A*, then the proportion of *Solution B* is increased linearly from 0% to 14.5% over 9.5 minutes, then increased from 14.5% to 100% over 7.5 minutes, and held

at 100% for an additional 1.5 minutes. Finally, the composition of the *Mobile phase* is changed to 100% *Solution A*, and is allowed to re-equilibrate for about 4 minutes or until a stable baseline is obtained. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for cefaclor and 1.0 for loracarbef; the resolution, R , between the cefaclor peak and the loracarbef peak is between 4.0 and 8.0; and the tailing factor for the loracarbef peak is not more than 1.3. Calculate the recovery of loracarbef from the *System suitability solution* by the formula:

$$100(C/L)(r_L/r_S)$$

in which C is the concentration, in mg per mL, of USP Loracarbef RS in the *Standard solution*; L is the concentration, in mg per mL, of USP Loracarbef RS in the *System suitability solution*; and r_L and r_S are the loracarbef responses in the chromatograms obtained from the *System suitability solution* and the *Standard solution*, respectively: the recovery is between 95% and 105%.

Procedure—Separately inject equal volumes (about 20 μ L) of *Solution A*, the *Phenylglycine solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Disregard any peak responses in the chromatograms that correspond to those in the chromatogram obtained from *Solution A*, and identify any peak in the chromatogram of the *Test solution* that corresponds to the peak for phenylglycine in the chromatogram obtained from the *Phenylglycine solution*. Separately calculate the percentage of each related compound in the portion of Loracarbef taken by the formula:

$$100(C/Y)(r_Y/r_S)$$

in which Y is the concentration, in mg per mL, of Loracarbef in the *Test solution*; r_Y is the response of any related compound in the chromatogram obtained from the *Test solution*; and C and r_S are as defined above: not more than 0.15% of phenylglycine is found, not more than 0.5% of any other related compound is found, and the sum of all other related compounds is not more than 2.0%.

Assay—

Mobile phase—Dissolve 2.0 g of sodium 1-pentanesulfonate in 1560 mL of water, add 20 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.5. Add 440 mL of methanol, mix, and pass through a filter having a porosity of 0.5 μ m or finer, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Transfer about 10 mg of USP Loracarbef RS, accurately weighed, to a 50-mL volumetric flask, dissolve in *Mobile phase*, using sonication, if necessary, to achieve dissolution, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer about 10 mg of Loracarbef, accurately weighed, to a 50-mL volumetric flask, dissolve in *Mobile phase*, using sonication, if necessary, to achieve dissolution, dilute with *Mobile phase* to volume, and mix.

Resolution solution—Prepare a solution in *Mobile phase* containing about 0.2 mg each of USP Loracarbef RS and of USP Loracarbef L-Isomer RS in each mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 265-nm detector and a 4.6-mm \times 25-cm column that contains 5- μ m packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the relative retention times are about 0.6 for loracarbef L-isomer and 1.0 for loracarbef; and the resolution, R , between the loracarbef L-isomer peak and the loracarbef peak is not less than 6.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the capacity factor for the loracarbef peak is not less than 5 and not more than 8; the tailing factor is

not less than 0.8 and not more than 1.3; the column efficiency, determined from the loracarbef peak, is not less than 2500 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in μ g, of anhydrous loracarbef ($C_{16}H_{16}ClN_3O_4$) in each mg of the Loracarbef taken by the formula:

$$(WP/w)(r_U/r_S)$$

in which W is the quantity, in mg, of USP Loracarbef RS taken to prepare the *Standard preparation*; P is the assigned potency, in μ g of anhydrous loracarbef ($C_{16}H_{16}ClN_3O_4$) in each mg of USP Loracarbef RS; w is the quantity, in mg, of Loracarbef taken to prepare the *Assay preparation*; and r_U and r_S are the loracarbef peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Loracarbef Capsules

» Loracarbef Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous loracarbef ($C_{16}H_{16}ClN_3O_4$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Cefaclor RS

USP Loracarbef RS

USP Loracarbef L-Isomer RS

Identification—The retention time of the loracarbef peak in the chromatogram of the *Assay preparation*, corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of anhydrous loracarbef ($C_{16}H_{16}ClN_3O_4$) dissolved from UV absorbances at the wavelength of maximum absorption at about 260 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Loracarbef RS in the same medium.

Tolerances—Not less than 75% (Q) of the labeled amount of anhydrous loracarbef ($C_{16}H_{16}ClN_3O_4$) is dissolved in 30 minutes.

Uniformity of dosage units (905)—meet the requirements.

Water Determination, Method I (921): not more than 8.5%.

Related compounds—

Solution A, *Solution B*, *Mobile phase*, *System suitability solution*, *Standard solution*, and *Chromatographic system*—Proceed as directed in the test for *Related compounds* under *Loracarbef*.

Test solution—Remove, as completely as possible, the contents of not less than 5 Capsules. Weigh the contents, and determine the average weight of the content of each Capsule. Transfer an accurately weighed portion of the powder, equivalent to 125 mg of loracarbef, based on the la-

beled amount per Capsule, to a 25-mL volumetric flask. Add about 20 mL of *Solution A* to the flask, mix, sonicate, and mix on a vortex mixer to aid in dissolution. Dilute with *Solution A* to volume, and mix. Filter, and use the filtrate as the *Test solution* immediately, or refrigerate and use within 24 hours.

Procedure—Proceed as directed for *Procedure* in the test for *Related compounds* under *Loracarbef*, except to omit the injection of *Phenylglycine solution*. Calculate the percentage of each related compound in the portion of Capsule contents taken by the formula:

$$100(C/Y)(r_1 / r_3)$$

in which *C* is the concentration, in mg per mL, of USP Loracarbef RS in the *Standard solution*; *Y* is the concentration, in mg per mL, of loracarbef in the *Test solution*; *r*₁ is the response of any related compound obtained from the *Test solution*; and *r*₃ is the loracarbef response obtained from the *Standard solution*: not more than 1.0% of any individual related compound is found, and the sum of all related compounds is not more than 3.0%.

Assay—

Mobile phase, Standard preparation, Resolution solution, and Chromatographic system—Proceed as directed in the Assay under *Loracarbef*.

Assay preparation—Remove, as completely as possible, the contents of not less than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 10 mg of loracarbef, to a 50-mL volumetric flask. Add about 40 mL of *Mobile phase*, and dissolve with the aid of swirling and sonication. Dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter having a porosity of 0.5 μm or finer, and use the filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the Assay under *Loracarbef*. Calculate the quantity, in mg, of loracarbef (C₁₆H₁₆ClN₃O₄) in the portion of Capsules taken by the formula:

$$(CP/20)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Loracarbef RS in the *Standard preparation*; *P* is the specified potency, in μg of anhydrous loracarbef (C₁₆H₁₆ClN₃O₄) per mg, of USP Loracarbef RS; and *r*_U and *r*_S are the loracarbef peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Loracarbef for Oral Suspension

» Loracarbef for Oral Suspension is a dry mixture of Loracarbef and one or more suitable suspending agents, preservatives, coloring agents, antifoaming agents, flavorings, and sweeteners. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of anhydrous loracarbef (C₁₆H₁₆ClN₃O₄).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Cefaclor RS

USP Loracarbef RS

USP Loracarbef L-Isomer RS

Identification—The retention time of the loracarbef peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

Uniformity of dosage units (905)—

FOR SOLIDS PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698): meets the requirements.

pH (791): between 3.0 and 5.5, in the Loracarbef for Oral Suspension constituted as directed in the labeling.

Water Determination, Method I (921): not more than 2.0%.

Related compounds—

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, and Chromatographic system—Proceed as directed in the test for *Related compounds* under *Loracarbef*.

Test solution—Constitute a container of Loracarbef for Oral Suspension as directed in the labeling. Transfer an accurately measured portion of the Suspension thus obtained, equivalent to 100 mg of loracarbef, based on the labeled amount per mL of the Suspension, to a 25-mL volumetric flask. Add about 20 mL of *Solution A* to the flask, mix, sonicate, and mix on a vortex mixer to effect dissolution. Dilute with *Solution A* to volume, and mix. Filter, and use the filtrate as the *Test solution* immediately, or refrigerate and use within 24 hours.

Procedure—Proceed as directed for *Procedure* in the test for *Related compounds* under *Loracarbef*, except to omit the injection of the *Phenylglycine solution*. Calculate the percentage of each related compound in the Suspension taken by the formula:

$$100(C/Y)(r_1 / r_3)$$

in which *C* is the concentration, in mg per mL, of USP Loracarbef RS in the *Standard solution*; *Y* is the concentration, in mg per mL, of loracarbef in the *Test solution*; *r*₁ is the response of any related compound obtained from the *Test solution*; and *r*₃ is the loracarbef response obtained from the *Standard solution*: not more than 1.0% of any individual related compound is found, and the sum of all related compounds is not more than 4.0%.

Assay—

Mobile phase, Standard preparation, Resolution solution, and Chromatographic system—Proceed as directed in the Assay under *Loracarbef*.

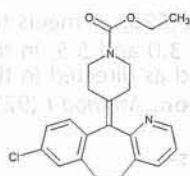
Assay preparation—Constitute 1 container of Loracarbef for Oral Suspension as directed in the labeling. Transfer an accurately measured volume of Loracarbef for Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 200 mg of Loracarbef, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter having a porosity of 0.5 μm or finer, and use the filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the Assay under *Loracarbef*. Calculate the quantity, in mg, of anhydrous loracarbef (C₁₆H₁₆ClN₃O₄) in each mL of Loracarbef for Oral Suspension taken by the formula:

$$(CP / V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Loracarbef RS in the *Standard preparation*; *P* is the specified potency, in μg of anhydrous loracarbef (C₁₆H₁₆ClN₃O₄) per mg, of USP Loracarbef RS; *V* is the volume, in mL, of Loracarbef for Oral Suspension taken to prepare the *Assay preparation*; and *r*_U and *r*_S are the loracarbef peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Loratadine



$C_{22}H_{23}ClN_2O_2$ 382.88

1-Piperidinecarboxylic acid, 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-, ethyl ester;

Ethyl 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidinecarboxylate [79794-75-5].

DEFINITION

Loratadine contains NLT 98.5% and NMT 101.0% of loratadine ($C_{22}H_{23}ClN_2O_2$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Buffer A (0.01 M dibasic potassium phosphate):

1.74 g/L of anhydrous dibasic potassium phosphate in water

Buffer B (0.6 M dibasic potassium phosphate): 105 g/L of anhydrous dibasic potassium phosphate in water

0.05 N hydrochloric acid: Transfer 500 mL of water to a 1000-mL volumetric flask, add 83 mL of hydrochloric acid, and dilute with water to volume. Transfer 50 mL of this solution into a 1000-mL volumetric flask, and dilute with water to volume.

Mobile phase: Acetonitrile, methanol, and *Buffer A* (60:60:70). Adjust with 10% phosphoric acid to an apparent pH of 7.2.

Diluent: Transfer 400 mL of 0.05 N hydrochloric acid and 80 mL of *Buffer B* to a 1-L volumetric flask. Dilute with a mixture of acetonitrile and methanol (1:1) to volume.

Standard solution: 0.4 mg/mL of USP Loratadine RS in *Diluent*

Sample solution: 0.4 mg/mL of Loratadine in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 25°–35°

Flow rate: 1 mL/min

Injection volume: 15 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of loratadine ($C_{22}H_{23}ClN_2O_2$) in the portion of Loratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): 10 ppm • (Official 1-Jan-2018)

ORGANIC IMPURITIES, PROCEDURE 1

[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* is recommended if 4,8-dichloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-one is a potential related compound.]

Mobile phase and Diluent: Proceed as directed in the Assay.

Standard solution: 0.8 μg/mL of USP Loratadine RS in *Diluent*

Sample solution: 0.4 mg/mL of Loratadine in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 25°–35°

Flow rate: 1 mL/min

Injection volume: 50 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 4.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Loratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of loratadine from the *Standard solution*

C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)

C_U = concentration of Loratadine in the *Sample solution* (mg/mL)

F = relative response factor as listed in *Table 1*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fluoroloratadine ^a	0.79	0.25	0.2
Loratadine	1.0	—	—
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	0.3

^a Ethyl 4-(8-chloro-11-fluoro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-yl) piperidin-1-carboxylate.

ORGANIC IMPURITIES, PROCEDURE 2

Solution A: Dissolve 0.96 g of 1-pentanesulfonic acid sodium salt in 900 mL of water. Adjust with phosphoric acid solution (1 in 10) to a pH of 3.00 ± 0.05, and dilute with water to 1 L.

Solution B: Acetonitrile

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	75	25
20	50	50
30	40	60
35	30	70
45	30	70
50	75	25

Standard stock solution: 0.1 mg/mL each of USP Loratadine RS, USP Loratadine Related Compound A RS, and USP Loratadine Related Compound B RS in methanol

Standard solution: 0.01 mg/mL each of USP Loratadine RS, USP Loratadine Related Compound A RS, and USP Loratadine Related Compound B RS prepared as follows. Transfer 1.0 mL of the *Standard stock solution* to a 10-mL volumetric flask, add 2 mL of *Solution A*, and dilute with methanol to volume.

Sample solution: 10 mg/mL of Loratadine prepared as follows. Transfer 100 mg of Loratadine to a 10-mL volumetric flask, and dissolve in 2 mL of methanol. Add 2 mL of *Solution A*, and then dilute with methanol to volume.

Chromatographic system
(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between loratadine related compound A and loratadine related compound B

Relative standard deviation: NMT 10% for the loratadine peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Loratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*

r_S = peak area of loratadine from the *Standard solution*

C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)

C_U = concentration of Loratadine in the *Sample solution* (mg/mL)

F = relative response factor as listed in Table 3.

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Loratadine related compound A	0.50	1.00	0.1
Loratadine related compound B	0.53	0.89	0.1
Loratadine related compound C ^a	0.70	0.60	0.1
Hydroxy deacyl analog ^b	0.75	0.46	0.1
Loratadine	1.00	—	—
Dichlorobenzo-cycloheptapyridine ^c	1.23	0.92	0.1
Hydroxyloratadine ^d	1.60	0.42	0.1
4-Chloroloratadine ^e	1.83	1.08	0.1
Any individual unknown impurity	—	1.0	0.10
Total impurities	—	—	0.3

^a 8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-one.

^b 8-Chloro-5,6-dihydro-11-hydroxy-11-(1-methylpiperidin-4-yl)-11H-benzo[5,6]cyclohepta[1,2-b]pyridine.

^c 4,8-Dichloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-one.

^d Ethyl 4-(8-chloro-11-hydroxy-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-yl) piperidin-1-carboxylate.

^e Ethyl 4-(4,8-dichloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)piperidin-1-carboxylate.

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry a sample at 100° to constant weight.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store between 2° and 30°.

• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.

• USP REFERENCE STANDARDS (11)

USP Loratadine RS

USP Loratadine Related Compound A RS

8-Chloro-5,6-dihydro-11-(piperidin-4-ylidene)-11H-benzo[5,6]cyclohepta[1,2-b]pyridine.

C₁₉H₁₉ClN₂ 310.82

USP Loratadine Related Compound B RS

8-Chloro-5,6-dihydro-11-(N-methylpiperidin-4-ylidene)-11H-benzo[5,6]cyclohepta[1,2-b]pyridine.

C₂₀H₂₁ClN₂ 324.85

Loratadine Oral Solution

DEFINITION

Loratadine Oral Solution contains NLT 94.0% and NMT 105.0% of the labeled amount of loratadine (C₂₂H₂₃ClN₂O₂).

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

Standard solution: 5 mg/mL of USP Loratadine RS in dichloromethane

Sample solution: Place a volume of Oral Solution, equivalent to 10 mg of loratadine, in a centrifuge tube. Add 10 mL of 0.2 N sodium hydroxide and 2.0 mL of dichloromethane. Rotate the centrifuge tube for 10 min, centrifuge, and discard the aqueous phase.

Developing solvent system: Ethyl ether and diethylamine (40:1), in a paper-lined tank

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Buffer: 6.8 g/L of monobasic potassium phosphate in water, adjusted with phosphoric acid to a pH of 3.0 ± 0.1

Mobile phase: Acetonitrile and *Buffer* (3:7)

Diluent: Acetonitrile and water (3:7)

Internal standard solution: 0.3 mg/mL of butylparaben in *Diluent*

Standard stock solution: 1.0 mg/mL of USP Loratadine RS in acetonitrile

Standard solution: Transfer 5.0 mL of *Internal standard solution*, 5.0 mL of *Standard stock solution*, and 12 mL of water to a 50-mL volumetric flask. Dilute with *Diluent* to volume.

Sample solution: Transfer a portion of Oral Solution, nominally equivalent to 5 mg of loratadine, to a 50-mL volumetric flask. Pipet 5.0 mL of *Internal standard solution* into the flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L11

Column temperature: 20°–30°

Flow rate: 2 mL/min

Injection size: 10 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for butylparaben and loratadine are about 0.78 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.9 between loratadine and butylparaben

Tailing factor: NMT 1.6 for the loratadine and butylparaben peaks

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of loratadine to the internal standard from the *Sample solution*

R_S = peak response ratio of loratadine to the internal standard from the *Standard solution*

C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)

Acceptance criteria: 94.0%–105.0%

PERFORMANCE TESTS

- **DELIVERABLE VOLUME** (698): Meets the requirements

IMPURITIES

ORGANIC IMPURITIES

Mobile phase: 4.3 g/L of sodium dodecyl sulfate in a mixture of acetonitrile and water (1:1). Adjust with phosphoric acid to a pH of 2.6 ± 0.1 .

Diluent: *Mobile phase* and water (2:1)

System suitability solution 1: 2 μ g/mL of USP Loratadine RS in *Diluent*

System suitability solution 2: 0.2 μ g/mL of USP Loratadine RS in *Diluent* from *System suitability solution 1*

System suitability solution 3: Transfer an amount of Oral Solution, equivalent to 20 mg of loratadine, into a screw-cap glass container. Add 1 mL of 3% aqueous hydrogen peroxide, and mix. Cap, and heat at 65° for 18–24 h. Allow to cool to room temperature, and then dilute 5 mL with *Diluent* to 25 mL.

Sample solution: 0.2 mg/mL of loratadine from a volume of Oral Solution in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Column temperature: 30°–40°

Flow rate: 2 mL/min

Injection size: 50 μ L

System suitability

Samples: *System suitability solution 1*, *System suitability solution 2*, and *System suitability solution 3*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between loratadine and 2-hydroxymethyl loratadine, *System suitability solution 3*

Tailing factor: 0.7–1.1, *System suitability solution 1*

Relative standard deviation: NMT 10%, *System suitability solution 2*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual related compound in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = individual peak response of each related compound in the *Sample solution*

r_T = sum of all the peak responses in the *Sample solution*, excluding excipient peaks

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
4-Hydroxymethyl loratadine ^a	0.70	0.3
2-Hydroxymethyl loratadine ^b	0.84	0.3
Loratadine	1.0	—
Any other individual impurity	—	0.2
Total impurities	—	0.5

^a Ethyl 4-[8-chloro-5,6-dihydro-4-(hydroxymethyl)-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene]-1-piperidinecarboxylate.

^b Ethyl 4-[8-chloro-5,6-dihydro-2-(hydroxymethyl)-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene]-1-piperidinecarboxylate.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count is NMT 10^2 cfu/mL, and the total combined molds and yeasts count is NMT 5×10^1 cfu/mL. It meets the requirements for the absence of *Salmonella* species and *Escherichia coli*.
- **PH** (791): 2.2–3.1

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 2° and 25°.
- **USP REFERENCE STANDARDS** (11)
USP Loratadine RS

Loratadine Tablets

DEFINITION

Loratadine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$).

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

Diluent: Chloroform and methanol (1:1)

Standard solution: 4 mg/mL of USP Loratadine RS in Diluent

Sample solution: Nominally 4 mg/mL of loratadine prepared as follows. Transfer a quantity of Tablets equivalent to 20 mg of loratadine to a centrifuge tube. Add 5.0 mL of Diluent, rotate for 30 min, and centrifuge.

Application volume: 5 μ L

Developing solvent system: Ethyl ether and diethylamine (40:1), in a paper-lined tank

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

ASSAY

• PROCEDURE

Buffer A: 0.01 M dibasic potassium phosphate (1.74 g/L of anhydrous dibasic potassium phosphate in water)

Buffer B: 0.6 M dibasic potassium phosphate (105 g/L of anhydrous dibasic potassium phosphate in water)

0.05 N hydrochloric acid: Transfer 500 mL of water to a 1000-mL volumetric flask, add 83 mL of hydrochloric acid, and dilute with water to volume. Transfer 50 mL of this solution to a 1000-mL volumetric flask, and dilute with water to volume.

Mobile phase: Acetonitrile, methanol, and Buffer A (60:60:70). Adjust with 10% phosphoric acid to a pH of 7.2.

Diluent: Transfer 400 mL of 0.05 N hydrochloric acid and 80 mL of Buffer B to a 1-L volumetric flask. Dilute with a mixture of acetonitrile and methanol (1:1) to volume.

Standard solution: 0.4 mg/mL of USP Loratadine RS in Diluent

Sample solution: Transfer 10 Tablets to a 250-mL volumetric flask, add 100 mL of 0.05 N hydrochloric acid, and shake for 40 min or until the Tablets are completely disintegrated. Add 75 mL of a mixture of acetonitrile and methanol (1:1) and 20 mL of Buffer B, and mix for 5 min. Dilute with a mixture of acetonitrile and methanol (1:1) to volume.

Chromatographic system
(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 25°–35°

Flow rate: 1 mL/min

Injection volume: 15 μ L

System suitability

Sample: Standard solution

Suitability requirements

Capacity factor: NLT 3.5

Tailing factor: NMT 1.7

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the Sample solution

r_s = peak response from the Standard solution

C_s = concentration of USP Loratadine RS in the Standard solution (mg/mL)

C_u = nominal concentration of loratadine in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Standard solution: USP Loratadine RS at a known concentration in Medium

Sample solution: A filtered portion of the solution under test, suitably diluted with Medium, if necessary

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: Maximum absorbance at about 280 nm

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) dissolved:

$$\text{Result} = (A_u/A_s) \times C_s \times D \times V \times (1/L) \times 100$$

A_u = absorbance of the Sample solution

A_s = absorbance of the Standard solution

C_s = concentration of USP Loratadine RS in the Standard solution (mg/mL)

D = dilution factor for the Sample solution, if needed

V = volume of Medium, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Buffer A, Buffer B, 0.05 N hydrochloric acid, Mobile phase, Diluent, and Sample solution: Proceed as directed in the Assay.

Standard solution: 0.8 μ g/mL of USP Loratadine RS in Diluent

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Flow rate: 1 mL/min

Injection volume: 50 μ L

System suitability

Sample: Standard solution

Suitability requirements

Relative standard deviation: NMT 4.0%

Analysis

Samples: Sample solution and Standard solution

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak area for each impurity from the Sample solution

r_s = peak area of loratadine from the Standard solution

C_s = concentration of USP Loratadine RS in the Standard solution (mg/mL)

C_u = nominal concentration of loratadine in the Sample solution (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fluoroloratadine ^{a,b}	0.79	—
Loratadine	1.0	—
Any other individual impurity	—	0.1
Total impurities	—	0.1

^aThis is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.

^bEthyl 4-(8-chloro-11-fluoro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-yl) piperidin-1-carboxylate.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 2° and 30°. Protect from excessive moisture if packaged in blisters.
- **USP REFERENCE STANDARDS (11)**
USP Loratadine RS

Loratadine Chewable Tablets

DEFINITION

Loratadine Chewable Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

Diluent: Chloroform and methanol (1:1)

Standard solution: 5 mg/mL of USP Loratadine RS in Diluent

Sample solution: Transfer a quantity of ground Chewable Tablets, equivalent to 25 mg of loratadine, to a centrifuge tube. Add 5 mL of Diluent, mix for 30 min, then centrifuge for 5 min. Use the clear supernatant.

Application volume: 5 μ L

Developing solvent system: Ethyl ether and diethylamine (40:1)

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

ASSAY

- **PROCEDURE**

Protect solutions containing loratadine from light.

Buffer A (phosphate buffer pH 7.2): Dissolve 4.35 g of dibasic potassium phosphate in 950 mL of water. Add 1 mL of triethylamine, adjust with 10% phosphoric acid or 10% potassium hydroxide to a pH of 7.2, and dilute with water to 1 L.

Buffer B (0.6 M dibasic potassium phosphate): 105 g/L of dibasic potassium phosphate in water

Diluent: Transfer 400 mL of 0.05 N hydrochloric acid and 80 mL of Buffer B to a 1-L volumetric flask. Dilute with a mixture of acetonitrile and methanol (1:1) to volume.

Mobile phase: Acetonitrile, methanol and Buffer A (40:10:50)

Standard solution: 0.4 mg/mL of USP Loratadine RS in Diluent

Sample solution: Nominally 0.4 mg/mL of loratadine in Diluent. Prepare by transferring 20 Chewable Tablets to a suitable volumetric flask. Add Diluent to 40% of the

volume of the flask, and shake for 40 min. Dilute with Diluent to volume, and filter.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 15 μ L

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 1.7

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) in the portion of Chewable Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Loratadine RS in the Standard solution (mg/mL)

C_U = nominal concentration of loratadine in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION (711)**

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 30 min

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 10% phosphoric acid to a pH of 2.80 ± 0.05 .

Mobile phase: Acetonitrile, methanol, and Buffer (40:30:35)

Standard solution: 10 μ g/mL of USP Loratadine RS in Medium

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 1.7

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Loratadine RS in the Standard solution (mg/mL)

L = label claim (mg/Tablet)

V = volume of Medium, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES**• ORGANIC IMPURITIES**

Protect solutions containing loratadine from light.

Mobile phase, Diluent, and Sample solution: Prepare as directed in the Assay.

System suitability solution: 0.8 µg/mL each of USP Loratadine Related Compound A RS, USP Loratadine Related Compound B RS, and USP Loratadine Related Compound C RS in *Diluent*.

Standard solution: 0.8 µg/mL of USP Loratadine RS in *Diluent*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Relative standard deviation: NMT 10%, *Standard solution*

Resolution: NLT 5.5 between loratadine related compound A and loratadine related compound C; NLT 2.0 between loratadine related compound C and loratadine related compound B, *System suitability solution*

Tailing factor: NMT 2.0 for loratadine related compound A, *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Chewable Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each impurity from the *Sample solution*

r_s = peak response of loratadine from the *Standard solution*

C_s = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of loratadine in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Reporting level for impurities: 0.05%

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Loratadine related compound A	0.20	1.0	0.05
Loratadine related compound C	0.32	0.63	0.10
Loratadine related compound B*	0.39	1.0	—
Loratadine	1.0	—	—

* This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.

Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.5

* This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store between 20° and 25°.

• **LABELING:** Label it to indicate that the Chewable Tablets are to be chewed before swallowing.

• USP REFERENCE STANDARDS (11)

USP Loratadine RS

USP Loratadine Related Compound A RS

8-Chloro-5,6-dihydro-11-(piperidin-4-ylidene)-11H-benzo[5,6]cyclohepta[1,2-b]pyridine.

$C_{19}H_{19}ClN_2$ 310.82

USP Loratadine Related Compound B RS

8-Chloro-5,6-dihydro-11-(N-methylpiperidin-4-ylidene)-11H-benzo[5,6]cyclohepta[1,2-b]pyridine.

$C_{20}H_{21}ClN_2$ 324.85

USP Loratadine Related Compound C RS

8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-one.

$C_{14}H_{10}ClNO$ 243.69

Loratadine Orally Disintegrating Tablets**DEFINITION**

Loratadine Orally Disintegrating Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

[NOTE—Matrix effects have been observed that affect the extraction of loratadine. Depending on the composition of the Tablet, use *Assay, Procedure 1* or *Procedure 2*.]

• PROCEDURE 1

Buffer: 2.72 g/L of monobasic potassium phosphate in water. Adjust with 5 N sodium hydroxide solution to a pH of 6.50 ± 0.05, and filter.

Mobile phase: Acetonitrile and *Buffer* (70:30)

Diluent: Acetonitrile and *Buffer* (40:60)

Standard solution: 0.1 mg/mL of USP Loratadine RS in *Mobile phase*

Sample solution: Transfer 10 Tablets into a 500-mL volumetric flask, add 400 mL of acetonitrile, and stir for 10 min. Sonicate the solution for 10 min, and stir for another 10 min. Dilute with acetonitrile to volume, and mix. Dilute an aliquot of the resulting solution with *Diluent* to obtain a solution having a concentration of about 0.1 mg/mL, based on the label claim. Pass a portion of this solution through a PVDF filter of 0.45-µm pore size, and discard the first 5 mL of filtrate.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L1**Flow rate:** 1.0 mL/min**Injection size:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Column efficiency:** NLT 3000 theoretical plates**Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of loratadine in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of loratadine from the *Sample solution* r_S = peak response of loratadine from the *Standard solution* C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL) C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)**Acceptance criteria:** 95.0%–105.0%**PROCEDURE 2****Buffer:** 2.28 g/L of dibasic potassium phosphate trihydrate**Mobile phase:** Methanol, acetonitrile, and *Buffer* (6:6:7), adjusted with 10% phosphoric acid to an apparent pH of 7.2**Diluent:** Methanol and water (1:1)**System suitability solution:** 0.8 μg/mL each of USP Loratadine Related Compound A RS, USP Loratadine Related Compound B RS, and USP Loratadine Related Compound C RS in *Diluent***Standard solution:** 0.4 mg/mL of USP Loratadine RS in *Diluent*. [NOTE—The solution may be sonicated for 5 min to aid in dissolving.]**Sample solution:** Transfer a number of Tablets into a 250-mL volumetric flask so that the final concentration is 0.4 mg/mL, based on the label claim. Add 50 mL of water, and sonicate, if necessary, to disperse the Tablets. Add 50 mL of methanol, and shake to dissolve. Dilute with *Diluent* to volume.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L7**Flow rate:** 1.0 mL/min**Injection size:** 15 μL for the *Standard solution* and *Sample solution*; 50 μL for the *System suitability solution***System suitability****Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for loratadine related compound A, loratadine related compound C, loratadine related compound B, and loratadine are about 0.26, 0.31, 0.42, and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 1.2 between loratadine related compound A and loratadine related compound C and NLT 1.2 between loratadine related compound C and loratadine related compound B, *System suitability solution***Tailing factor:** NMT 2.0, *Standard solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of loratadine in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of loratadine from the *Sample solution* r_S = peak response of loratadine from the *Standard solution* C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL) C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)**Acceptance criteria:** 95.0%–105.0%**PERFORMANCE TESTS****• DISINTEGRATION (701)****Test 1****Stage 1:** All 6 Tablets completely disintegrate in 1 min.**Stage 2:** NLT 16 of 18 Tablets completely disintegrate in 1 min.**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Disintegration Test 2*.**Analysis:** Place a stainless steel wire clip on each Tablet to prevent the Tablet from floating.**Acceptance criteria:** NMT 30 s**• DISSOLUTION (711)****Medium:** Simulated gastric fluid without enzymes; 900 mL, deaerated**Apparatus 1:** 50 rpm**Time:** 6 min**Standard solution:** Prepare a solution of USP Loratadine RS in *Medium* at a concentration similar to that expected in the *Sample solution*.**Sample solution:** Pass a portion of the solution under test through a suitable filter.**Analysis****Detector:** UV 278 nm**Cell length:** 1 cm**Blank:** *Medium*Calculate the percentage of the labeled amount of loratadine ($C_{22}H_{23}ClN_2O_2$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/L) \times 100$$

 A_U = absorbance from the *Sample solution* A_S = absorbance from the *Standard solution* C_S = concentration of the *Standard solution* (mg/mL) L = label claim (mg/Tablet) V = volume of *Medium*, 900 mL**Tolerances:** NLT 80% (Q) of the labeled amount of loratadine is dissolved.**• UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**IMPURITIES****Organic Impurities****• PROCEDURE 1**[NOTE—Use *Organic Impurities, Procedure 1* if *Assay, Procedure 1* is used.]**Buffer, Mobile phase, and Diluent:** Proceed as directed in *Assay, Procedure 1*.**System sensitivity solution:** 0.05 μg/mL of USP Loratadine RS in *Mobile phase***Standard solution:** 0.5 μg/mL of USP Loratadine RS in *Mobile phase***Sample solution:** Transfer 10 Tablets into a 500-mL volumetric flask, add 400 mL of acetonitrile, and stir for about 10 min. Sonicate the solution for 10 min, and stir for another 10 min. Dilute with acetonitrile to volume, and mix. Dilute an aliquot of the resulting solution with *Diluent* to obtain a solution having a concen-

tration of about 0.1 mg/mL, based on the label claim. Centrifuge the solution for about 10 min, and use the supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1.0 mL/min

Injection size: 50 μL

System suitability

Samples: *System sensitivity solution* and *Standard solution*

Suitability requirements

Signal-to-noise ratio: NLT 10, *System sensitivity solution*

Column efficiency: NLT 3000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of loratadine from the *Standard solution*

C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Loratadine related compound C	0.5	0.64	0.2
Loratadine	1.0	—	—
Individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.3

PROCEDURE 2

[NOTE—Use *Organic Impurities, Procedure 2* if *Assay, Procedure 2* is used.]

Buffer, Mobile phase, Diluent, Sample solution, and System suitability solution: Proceed as directed in *Assay, Procedure 2*.

System sensitivity solution: 0.04 μg/mL of USP Loratadine RS in *Diluent*

Standard solution: 0.8 μg/mL of USP Loratadine RS in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Flow rate: 1.0 mL/min

Injection size: 50 μL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *System sensitivity solution*

[NOTE—The relative retention times for loratadine related compound A, loratadine related compound C, loratadine related compound B, and loratadine are about 0.26, 0.31, 0.42, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.2 between loratadine related compound A and loratadine related compound C and NLT 1.2 between loratadine related compound C and loratadine related compound B, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 4.0%, *Standard solution*

Signal-to-noise ratio: NLT 3.0, *System sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of loratadine from the *Standard solution*

C_S = concentration of USP Loratadine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of loratadine in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Loratadine related compound A	0.26	0.9	0.1
Loratadine related compound B ^a	0.42	—	—
Unspecified impurity ^a	0.76	—	—
Loratadine	1.0	—	—
Unspecified impurity ^a	1.5	—	—
Individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.1

^a These impurities are controlled in the drug substance and are listed here for information only. These impurities are not included when determining total impurities.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 20° and 25°.

• **LABELING:** The labeling states with which *Organic Impurities* and *Assay* procedure the article complies, if other than *Procedure 1*. When more than one *Disintegration* test is given, the labeling states the *Disintegration* test used only if *Test 1* is not used.

USP REFERENCE STANDARDS (11)

USP Loratadine RS

USP Loratadine Related Compound A RS

8-Chloro-5,6-dihydro-11-(piperidin-4-ylidene)-11H-benzo[5,6]cyclohepta[1,2-b]pyridine.

C₁₉H₁₉ClN₂ 310.82

USP Loratadine Related Compound B RS

8-Chloro-5,6-dihydro-11-(N-methylpiperidin-4-ylidene)-11H-benzo[5,6]cyclohepta[1,2-b]pyridine.

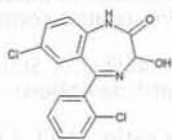
C₂₀H₂₁ClN₂ 324.85

USP Loratadine Related Compound C RS

8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-one.

C₁₄H₁₀ClNO 243.69

Lorazepam



$C_{15}H_{10}Cl_2N_2O_2$ 321.16
 2H-1,4-Benzodiazepin-2-one, 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-, (\pm);
 (\pm)-7-Chloro-5-(o-chlorophenyl)-1,3-dihydro-3-hydroxy-2H-1,4-benzodiazepin-2-one [846-49-1].

DEFINITION

Lorazepam contains NLT 98.0% and NMT 102.0% of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$), calculated on the dried basis.

IDENTIFICATION

- A. INFRARED ABSORPTION (197K)**
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Mobile phase: Acetonitrile, glacial acetic acid, and water (50:1.2:50)

Diluent: Methanol and water (75:25)

Standard solution: 0.1 mg/mL of USP Lorazepam RS in *Diluent*

Sample solution: 0.1 mg/mL of Lorazepam in *Diluent*

Chromatographic system
 (See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Temperatures

Column: 5°

Sample chamber: 4°

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Collect data for at least 50 min.

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$) in the portion of Lorazepam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lorazepam from the *Sample solution*

r_S = peak response of lorazepam from the *Standard solution*

C_S = concentration of USP Lorazepam RS in the *Standard solution* (mg/mL)

C_U = concentration of Lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- RESIDUE ON IGNITION (281):** NMT 0.3%

Delete the following:

- HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-

Jan-2018)

ORGANIC IMPURITIES

Mobile phase and Diluent: Prepare as directed in the *Assay*.

Standard solution: 32 μ g/mL of USP Lorazepam RS in *Diluent*

System suitability solution: 3.2 mg/mL of USP Lorazepam RS and 32 μ g/mL each of USP Lorazepam Related Compound A RS, USP Lorazepam Related Compound B RS, USP Lorazepam Related Compound C RS, USP Lorazepam Related Compound D RS, and USP Lorazepam Related Compound E RS in *Diluent*

Sample solution: 3.2 mg/mL of Lorazepam in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Temperatures

Column: 5°

Sample chamber: 4°

Flow rate: 1 mL/min

Injection volume: 100 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—See *Table 1* for the relative retention times.]

Collect data for at least 50 min.

Suitability requirements

Resolution: NLT 1.2 between lorazepam related compound A and lorazepam related compound E, *System suitability solution*

Tailing factor: NMT 2.0 for lorazepam, *Standard solution*

Relative standard deviation: NMT 5% for lorazepam, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Lorazepam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of lorazepam from the *Standard solution*

C_S = concentration of lorazepam in the *Standard solution* (mg/mL)

C_U = concentration of Lorazepam in the *Sample solution* (mg/mL)

F = relative response factor for any given impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lorazepam	1.0	1.0	—
Lorazepam related compound D ^a	1.4	1.0	0.15
Lorazepam related compound A ^b	1.7	1.0	0.10
Lorazepam related compound E ^c	1.9	1.3	0.15

^a 6-Chloro-4-(o-chlorophenyl)-2-quinazolinecarboxylic acid.

^b 7-Chloro-5-(o-chlorophenyl)-1,3-dihydro-3-acetoxy-2H-1,4-benzodiazepin-2-one.

^c 6-Chloro-4-(o-chlorophenyl)-2-quinazoline methanol.

^d 6-Chloro-4-(o-chlorophenyl)-2-quinazolinecarboxaldehyde.

^e 2-Amino-2',5'-dichlorobenzophenone.

Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lorazepam related compound C ^d	2.1	1.0	0.30
Lorazepam related compound B ^e	5.5	1.0	0.01
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.75

^a 6-Chloro-4-(o-chlorophenyl)-2-quinazolinecarboxylic acid.^b 7-Chloro-5-(o-chlorophenyl)-1,3-dihydro-3-acetoxy-2H-1,4-benzodiazepin-2-one.^c 6-Chloro-4-(o-chlorophenyl)-2-quinazoline methanol.^d 6-Chloro-4-(o-chlorophenyl)-2-quinazolinecarboxaldehyde.^e 2-Amino-2',5'-dichlorobenzophenone.**SPECIFIC TESTS****• LOSS ON DRYING (731)**

Sample: Dry under vacuum at 105° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Change to read:**• USP REFERENCE STANDARDS (11)**

USP Lorazepam RS

USP Lorazepam Related Compound A RS

7-Chloro-5-(o-chlorophenyl)-1,3-dihydro-3-acetoxy-2H-1,4-benzodiazepin-2-one.

C₁₇H₁₂Cl₂N₂O₃ 363.20

USP Lorazepam Related Compound B RS

2-Amino-2',5'-dichlorobenzophenone.

C₁₃H₉Cl₂NO • (ERR 1-Jun-2016) 266.13

USP Lorazepam Related Compound C RS

6-Chloro-4-(o-chlorophenyl)-2-quinazolinecarboxaldehyde.

C₁₅H₈Cl₂N₂O 303.15

USP Lorazepam Related Compound D RS

6-Chloro-4-(o-chlorophenyl)-2-quinazolinecarboxylic acid.

C₁₅H₈Cl₂N₂O₂ 319.15

USP Lorazepam Related Compound E RS

6-Chloro-4-(o-chlorophenyl)-2-quinazoline methanol.

C₁₅H₁₀Cl₂N₂O 305.16**Lorazepam Injection****DEFINITION**

Lorazepam Injection is a sterile solution of Lorazepam in a suitable medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of lorazepam (C₁₅H₁₀Cl₂N₂O₂).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. THIN-LAYER CHROMATOGRAPHY**
Standard stock solution: 1 mg/mL of USP Lorazepam RS in alcohol
Standard solution: Transfer 10 mL of *Standard stock solution* to a suitable container. Add 5 mL of hydrochloric acid, heat the solution on a steam bath for 20 min, and

cool. Transfer the solution to a separator, and add 8 mL of 10 N sodium hydroxide. Extract the solution with two 25-mL portions of ether, filtering the ether extracts through cotton plugs. Evaporate the ether extract to 2 mL, and add 8 mL of methanol.

Sample solution: Transfer a volume of *Injection*, equivalent to 10 mg of lorazepam, to a container. Add 5 mL of hydrochloric acid, heat on a steam bath for 20 min, and cool. Transfer the solution to a separator, and add 8 mL of 10 N sodium hydroxide. Extract with two 25-mL portions of ether, filtering the ether extracts through cotton plugs. Evaporate the ether extract to 2 mL, and add 8 mL of methanol.

Chromatographic system(See *Chromatography* (621), *Thin-Layer Chromatography*.)**Mode:** TLC**Adsorbent:** Thin-layer chromatographic plate, coated with a 0.25-mm layer of chromatographic silica gel**Application volume:** 10 µL**Developing solvent system:** Toluene**Spray reagent 1:** 12.5 mg/mL of sodium nitrite in 0.5 N hydrochloric acid, freshly prepared**Spray reagent 2:** 1 mg/mL of *N*-(1-naphthyl)ethylene-diamine dihydrochloride in alcohol**Analysis****Samples:** *Standard solution* and *Sample solution*

Allow the spots to dry, and develop the chromatograms until the solvent front has moved 15 cm. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with *Spray reagent 1*. Heat the plate at 100° for 5 min, allow to cool, and spray with *Spray reagent 2*.

Acceptance criteria: The *R_f* value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY**• PROCEDURE****Buffer:** 0.05 M monobasic ammonium phosphate**Mobile phase:** Methanol and *Buffer* (50:50). Adjust with ammonium hydroxide to a pH of 6.5.

System suitability solution: 0.04 mg/mL of lorazepam and 32 µg/mL each of USP Lorazepam Related Compound C RS and USP Lorazepam Related Compound D RS in *Mobile phase*

Standard stock solution: 1 mg/mL of USP Lorazepam RS in methanol**Standard solution:** 0.16 mg/mL of USP Lorazepam RS in *Mobile phase* from *Standard stock solution***Sample solution:** Nominally 0.16 mg/mL of lorazepam from *Injection*, diluted with *Mobile phase***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 240 nm**Column:** 4.6-mm × 10 to 15-cm; packing L1**Flow rate:** 2 mL/min**Injection volume:** 20 µL**System suitability****Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for lorazepam related compound D, lorazepam, and lorazepam related compound C are 0.7, 1.0, and 2.7, respectively.]

Suitability requirements

Resolution: NLT 1.2 between lorazepam related compound D and lorazepam and NLT 1.2 between lorazepam and lorazepam related compound C, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$) in the portion of Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- r_u = peak response of lorazepam from the *Sample solution*
 r_s = peak response of lorazepam from the *Standard solution*
 C_s = concentration of USP Lorazepam RS in the *Standard solution* (mg/mL)
 C_u = nominal concentration of lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES**• ORGANIC IMPURITIES**

Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution 1: Prepare as directed for the *Standard solution* in the *Assay*.

Standard solution 2: 3.2 µg/mL each of USP Lorazepam Related Compound C RS and USP Lorazepam Related Compound D RS in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution 1*

[NOTE—The relative retention times for lorazepam related compound D, lorazepam, and lorazepam related compound C are 0.7, 1.0, and 2.7, respectively.]

Suitability requirements

Resolution: NLT 1.2 between lorazepam related compound D and lorazepam and NLT 1.2 between lorazepam and lorazepam related compound C, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution 1*

Analysis

Samples: *Sample solution* and *Standard solution 2*. Do not include as an impurity any peak from the *Sample solution* that has a retention time shorter than that of the lorazepam related compound D peak from *Standard solution 2*.

Calculate the percentage of lorazepam related compound C and lorazepam related compound D in the portion of Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- r_u = peak response of lorazepam related compound C or lorazepam related compound D from the *Sample solution*
 r_s = peak response of the corresponding related compound from the *Standard solution*
 C_s = concentration of the corresponding related compound in the *Standard solution* (µg/mL)
 C_u = nominal concentration of lorazepam in the *Sample solution* (µg/mL)

Calculate the percentage of any other impurity in the portion of Injection taken:

$$\text{Result} = (r_u/r_r) \times 100$$

- r_u = peak response of the individual impurity from the *Sample solution*
 r_r = peak response of lorazepam from the *Sample solution*

Acceptance criteria

Total impurities: NMT 4.0% of all impurities

• LIMIT OF LORAZEPAM RELATED COMPOUND B

Standard solution: 0.1 mg/mL of USP Lorazepam Related Compound B RS in acetone

Sample solution: Nominally 10 mg/mL of lorazepam prepared as follows. To 5.0 mL of Injection in a separator add 50 mL of 0.1 N sodium hydroxide. Extract with three 10-mL portions of chloroform, and collect the chloroform extracts in a second separator. Wash the chloroform extracts with 10 mL of water, and transfer the chloroform extracts to a centrifuge tube. Evaporate the chloroform extracts with the aid of a current of air to dryness, and dissolve the residue in acetone.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: Thin-layer chromatographic plate, coated with a 0.25-mm layer of chromatographic silica gel

Application volume

Standard solution: 5 µL

Sample solution: 50 µL

Developing solvent system: Chloroform, *n*-heptane, and alcohol (10:10:1)

Spray reagent 1: 2 N sulfuric acid

Spray reagent 2: 1 mg/mL of sodium nitrite

Spray reagent 3: 5 mg/mL of ammonium sulfamate

Spray reagent 4: 1 mg/mL of *N*-(1-naphthyl)ethylene-diamine dihydrochloride

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter. Allow the spots to dry, and develop the chromatograms in the *Developing solvent system* until the solvent front has moved NLT 10 cm from the origin. Lightly spray the plate with *Spray reagent 1*, dry at 105° for 15 min, and spray successively with *Spray reagent 2*, *Spray reagent 3*, and *Spray reagent 4*, drying the plate with a current of air after each spraying. Observe the plate under visible light.

Acceptance criteria: The spot of the *Sample solution* is not greater in size or intensity than the principal spot at the corresponding R_f value of the *Standard solution*, corresponding to NMT 0.1% of lorazepam related compound B.

SPECIFIC TESTS

- BACTERIAL ENDOTOXINS TEST (85):** Contains NMT 10^2 USP Endotoxin Units/mg of lorazepam
- OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

Change to read:**• USP REFERENCE STANDARDS (11)**

USP Endotoxin RS

USP Lorazepam RS

USP Lorazepam Related Compound B RS

2-Amino-2',5-dichlorobenzophenone.

• $C_{13}H_9Cl_2NO$ (ERR 1-JUN-2016) 266.13

USP Lorazepam Related Compound C RS

6-Chloro-4-(*o*-chlorophenyl)-

2-quinazolinecarboxaldehyde.

$C_{15}H_8Cl_2N_2O$ 303.15

USP Lorazepam Related Compound D RS

6-Chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxylic acid.

$C_{15}H_8Cl_2N_2O_2$ 319.15

Lorazepam Oral Concentrate

DEFINITION

Lorazepam Oral Concentrate contains NLT 90.0% and NMT 110.0% of the labeled amount of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$).

IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Mobile phase: Acetonitrile, glacial acetic acid, and water (45:0.2:55)

Standard solution: 0.05 mg/mL of USP Lorazepam RS in methanol

System suitability solution: 0.1 mg/mL each of USP Lorazepam RS and USP Lorazepam Related Compound E RS in methanol

Sample solution: Nominally 0.05 mg/mL of lorazepam prepared as follows. Transfer a suitable volume of Oral Concentrate to a volumetric flask, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for lorazepam and lorazepam related compound E are 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between lorazepam and lorazepam related compound E, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$) in the portion of Oral Concentrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Lorazepam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

ORGANIC IMPURITIES

Mobile phase: Methanol and 0.05 M monobasic ammonium phosphate (64:36)

Diluent: Methanol and 0.05 M monobasic ammonium phosphate (50:50). Adjust with ammonium hydroxide to a pH of 6.5.

Standard stock solution: 1.0 mg/mL USP Lorazepam RS in methanol

Standard solution 1: 0.16 µg/mL of lorazepam from the *Standard stock solution* in *Diluent*

Standard solution 2: 0.16 µg/mL of USP Lorazepam Related Compound B RS, and 3.2 µg/mL each of USP

Lorazepam Related Compound C RS and USP

Lorazepam Related Compound D RS in *Mobile phase*

System suitability solution: 0.04 mg/mL of USP

Lorazepam RS, and 0.032 mg/mL each of USP

Lorazepam Related Compound C RS and USP

Lorazepam Related Compound D RS in *Diluent*

Sample solution: Nominally 0.16 mg/mL of lorazepam prepared as follows. Transfer a suitable volume of Oral Concentrate to a volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 10- to 15-cm; packing L1

Flow rate: 0.7 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution 1*

Suitability requirements

Resolution: NLT 1.2 between lorazepam related compound D and lorazepam; NLT 1.2 between lorazepam and lorazepam related compound C, *System suitability solution*

Relative standard deviation: NMT 2.0% for lorazepam, *Standard solution 1*

Analysis

Samples: *Standard solution 2* and *Sample solution*

[NOTE—Disregard peaks eluting prior to lorazepam related compound D.]

Calculate the percentage of lorazepam related compound B, lorazepam related compound C, and lorazepam related compound D in the portion of Oral Concentrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of lorazepam related compound B, lorazepam related compound C, or lorazepam related compound D from the *Sample solution*

r_S = peak response of the corresponding related compound from *Standard solution 2*

C_S = concentration of the corresponding related compound in *Standard solution 2* (mg/mL)

C_U = nominal concentration of lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lorazepam related compound D	0.8	4.0 ^a
Lorazepam	1.0	—
Lorazepam related compound C	2.3	4.0 ^a
Lorazepam related compound B	2.9	0.1

^a Includes the sum of lorazepam related compound C and lorazepam related compound D.

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

Change to read:• **USP REFERENCE STANDARDS (11)**

USP Lorazepam RS

USP Lorazepam Related Compound B RS

2-Amino-2',5-dichlorobenzophenone.

C₁₃H₉Cl₂NO • (ERR 1-Jun-2016) 266.13

USP Lorazepam Related Compound C RS

6-Chloro-4-(o-chlorophenyl)-

2-quinazolinecarboxaldehyde.

C₁₅H₈Cl₂N₂O 303.15

USP Lorazepam Related Compound D RS

6-Chloro-4-(o-chlorophenyl)-2-quinazolinecarboxylic acid.

C₁₅H₈Cl₂N₂O₂ 319.15

USP Lorazepam Related Compound E RS

6-Chloro-4-(o-chlorophenyl)-2-quinazoline methanol.

C₁₅H₁₀Cl₂N₂O 305.16**Lorazepam Tablets****DEFINITION**

Lorazepam Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lorazepam (C₁₅H₁₀Cl₂N₂O₂).

IDENTIFICATION• **A. INFRARED ABSORPTION (197M)**

Sample: Stir a portion of finely powdered Tablets, equivalent to 15 mg of lorazepam, with 40 mL of acetone for 5 min. Pass through very retentive filter paper pre-washed with acetone. Evaporate the filtrate to dryness on a steam bath with the aid of a current of air. Dissolve the residue in 1 mL of acetone, and add 20 mL of 2,2,4-trimethylpentane. Heat the solution on a hot plate to a gentle boil, and evaporate to a volume of about 10 mL. Remove the solution from the hot plate, and evaporate to dryness with the aid of a current of air. Dry the residue under vacuum at 60° for 1 h.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Diluent: Methanol and water (85:15)

Mobile phase: Acetonitrile, glacial acetic acid, and water (40:0.4:60)

Standard solution: 0.1 mg/mL of USP Lorazepam RS in *Diluent*

Sample solution: Nominally 0.1 mg/mL of lorazepam prepared as follows. Transfer 20 Tablets to a 100-mL volumetric flask, add 50 mL of *Diluent*, sonicate for 10 min, and shake by mechanical means for 20 min. Dilute with *Diluent* to volume, mix, and centrifuge a portion of the solution at 2000 rpm for 10 min. Dilute a portion of the clear supernatant with *Diluent*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lorazepam (C₁₅H₁₀Cl₂N₂O₂) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Lorazepam RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: Water; 500 mL

Apparatus 1: 100 rpm

Times: 30 and 60 min

Mobile phase and Chromatographic system: Prepare as directed in the *Assay*, except use an *Injection volume* of 50 μL.

Standard solution: USP Lorazepam RS at a known concentration in *Medium*. Initially, use a volume of alcohol not exceeding 10% of the final volume of the *Standard solution* to dissolve the Reference Standard.

Sample solution: *Sample per Dissolution (711)*.

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 60% (Q) of the labeled amount of lorazepam (C₁₅H₁₀Cl₂N₂O₂) is dissolved in 30 min. NLT 80% (Q) of the labeled amount of lorazepam (C₁₅H₁₀Cl₂N₂O₂) is dissolved in 60 min.

• **UNIFORMITY OF DOSAGE UNITS (905)**

Procedure for content uniformity

Diluent, Mobile phase, Standard solution, and Chromatographic system: Proceed as directed in the *Assay*.

Sample solution: Nominally, 0.1 mg/mL of lorazepam prepared as follows. Place 1 Tablet in a volumetric flask of appropriate size, based on the labeled quantity, in mg, of lorazepam in the Tablet. Add a volume of *Diluent* equal to about 50% of the volume of the flask, sonicate for 10 min, and shake by mechanical means for 20 min. Dilute with *Diluent* to volume, mix, and centrifuge a portion of the solution for 10 min at 2000 rpm.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of lorazepam (C₁₅H₁₀Cl₂N₂O₂) in the portion of the Tablet taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Lorazepam RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of lorazepam in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Buffer: 67.7 g/L of sodium acetate trihydrate in water.

Adjust with glacial acetic acid to a pH of 5.0 ± 0.05 .

Mobile phase: Acetonitrile, glacial acetic acid, and water (50:1.2:50)

Diluent: Methanol and Buffer (75:25)

Standard solution: 1.6 µg/mL of USP Lorazepam RS in Diluent

Peak identification solution: 0.16 mg/mL of USP Lorazepam RS, 1.6 µg/mL each of USP Lorazepam Related Compound A RS, USP Lorazepam Related Compound B RS, USP Lorazepam Related Compound C RS, USP Lorazepam Related Compound D RS, and USP Lorazepam Related Compound E RS in Diluent

Sample solution: Nominally 0.16 mg/mL of lorazepam prepared as follows. Transfer a weighed amount of lorazepam, equivalent to 21.3 mg from powdered Tablets, to a 25-mL volumetric flask. Add 20 mL of Diluent, and stir for 15 min. Do not dilute to volume. Centrifuge at 2000 rpm for 15 min. Pass the supernatant through a polyethersulfone membrane of 0.45-µm pore size. Dilute a portion of the filtrate with Diluent.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC. Use an instrument equipped with a sample compartment chiller maintained at 4°.

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 5°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: At least 50 min

System suitability

Samples: Standard solution and Peak identification solution

[NOTE—See Table 1 for the approximate relative retention times.]

Suitability requirements

Resolution: NLT 1.2 between lorazepam related compound A and lorazepam related compound E, Peak identification solution

Tailing factor: NMT 2.0, Standard solution

Relative standard deviation: NMT 5%, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the Sample solution

r_S = peak response for lorazepam from the Standard solution

C_S = concentration of lorazepam in the Standard solution (mg/mL)

C_U = nominal concentration of lorazepam in the Sample solution (mg/mL)

F = relative response factor for any given impurity (see Table 1)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lorazepam	1.0	1.0	—
Lorazepam related compound D ^a	1.4	1.0	0.5
Lorazepam related compound A ^{b,c}	1.7	—	—
Lorazepam related compound E ^d	1.9	1.3	0.5
Lorazepam related compound C ^e	2.1	1.0	3.0
Lorazepam related compound B ^f	5.5	1.0	0.1
Any individual unspecified degradation product	—	1.0	0.2
Total impurities	—	—	4.0

^a 6-Chloro-4-(o-chlorophenyl)-2-quinazolinecarboxylic acid.

^b 7-Chloro-5-(o-chlorophenyl)-1,3-dihydro-3-acetoxy-2H-1,4-benzodiazepin-2-one.

^c Lorazepam related compound A is included only for peak identification purposes. It is not quantified and should not be included in the total impurities calculation.

^d 6-Chloro-4-(o-chlorophenyl)-2-quinazoline methanol.

^e 6-Chloro-4-(o-chlorophenyl)-2-quinazolinecarboxaldehyde.

^f 2-Amino-2',5-dichlorobenzophenone.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Change to read:

• USP REFERENCE STANDARDS (11)

USP Lorazepam RS

USP Lorazepam Related Compound A RS

7-Chloro-5-(o-chlorophenyl)-1,3-dihydro-3-acetoxy-2H-1,4-benzodiazepin-2-one.

$C_{17}H_{12}Cl_2N_2O_3$ 363.20

USP Lorazepam Related Compound B RS

2-Amino-2',5-dichlorobenzophenone.

$C_{13}H_9Cl_2NO$ (ERR 1-Jun-2016) 266.13

USP Lorazepam Related Compound C RS

6-Chloro-4-(o-chlorophenyl)-2-quinazolinecarboxaldehyde.

$C_{15}H_8Cl_2N_2O$ 303.15

USP Lorazepam Related Compound D RS

6-Chloro-4-(o-chlorophenyl)-2-quinazolinecarboxylic acid.

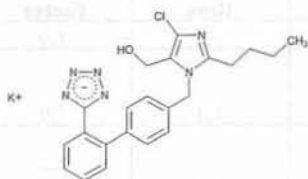
$C_{15}H_8Cl_2N_2O_2$ 319.15

USP Lorazepam Related Compound E RS

6-Chloro-4-(o-chlorophenyl)-2-quinazoline methanol.

$C_{15}H_{10}Cl_2N_2O$ 305.16

Losartan Potassium



$C_{22}H_{22}ClKN_6O$ 461.00
 1*H*-Imidazole-5-methanol, 2-butyl-4-chloro-1-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl-, monopotassium salt;
 2-Butyl-4-chloro-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]imidazole-5-methanol, monopotassium salt
 [124750-99-8].

DEFINITION

Losartan Potassium contains NLT 98.5% and NMT 101.0% of losartan potassium ($C_{22}H_{22}ClKN_6O$), calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

- A. INFRARED ABSORPTION** (197M) or (197K): Meets the requirements
- B. ULTRAVIOLET ABSORPTION** (197U)
 Sample solution: 10 µg/mL in methanol
 Acceptance criteria: Meets the requirements
- C. IDENTIFICATION TESTS—GENERAL, Potassium** (191): Meets the requirements

ASSAY

PROCEDURE

Solution A: 0.1% solution of phosphoric acid in water
Solution B: Acetonitrile
Mobile phase: *Solution B* and *Solution A* (2:3)
Standard solution: 0.25 mg/mL of USP Losartan Potassium RS in methanol
Sample solution: 0.25 mg/mL of Losartan Potassium in methanol

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC
Detector: UV 254 nm
Column: 4.0-mm × 25-cm; packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability

Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 5600 theoretical plates
Tailing factor: NMT 1.4
Relative standard deviation: NMT 0.5%

Analysis

Samples: *Standard solution* and *Sample solution*
 Calculate the percentage of losartan potassium ($C_{22}H_{22}ClKN_6O$) in the portion of Losartan Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
 r_S = peak area from the *Standard solution*
 C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)
 C_U = concentration of the *Sample solution* (mg/mL)
Acceptance criteria: 98.5%–101.0% on the anhydrous, solvent-free basis

IMPURITIES

Delete the following:

- HEAVY METALS, Method II (231):** NMT 10 ppm (Official 1: Jan-2018)
- ORGANIC IMPURITIES**
Solution A: 0.1% solution of phosphoric acid in water
Solution B: Acetonitrile
System suitability solution: 0.3 mg/mL of USP Losartan Potassium RS and 2 µg/mL of triphenylmethanol in methanol
Sample solution: 0.3 mg/mL of Losartan Potassium in methanol
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
25	10	90
35	10	90
45	75	25
50	75	25

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.0-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for losartan and triphenylmethanol are 1.0 and 1.9, respectively. The typical retention time for triphenylmethanol is 20 min.]

Suitability requirements

Tailing factor: NMT 1.6

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of losartan potassium ($C_{22}H_{22}ClKN_6O$) taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity
 r_T = sum of the responses for all peaks

Acceptance criteria

Individual impurities: NMT 0.2%

Total impurities: NMT 0.5%

SPECIFIC TESTS

- WATER DETERMINATION, Method I (921):** NMT 0.5%. If labeled as an amorphous form, NMT 5.0%.

ADDITIONAL REQUIREMENTS

- LABELING:** Where it is an amorphous form, the label so indicates.
- PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
 USP Losartan Potassium RS

Losartan Potassium Tablets

DEFINITION

Losartan Potassium Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$).

IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Buffer: 1.25 mg/mL of monobasic potassium phosphate and 1.5 mg/mL of dibasic sodium phosphate in water. The resulting pH is approximately 7.0. Pass the solution through a PTFE or equivalent filter of 0.45- μ m pore size, and degas before use.

Solution A: Acetonitrile and *Buffer* (15:85)

Solution B: Use acetonitrile.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
10	40	60
11	80	20
15	80	20

System suitability stock solution: Dissolve 12 mg of USP Losartan Potassium RS in a 50-mL volumetric flask, first using 5 mL of water, followed by 5 mL of 0.1 N hydrochloric acid. Place the flask in a 105° oven for 1–2 h, and allow to cool to room temperature. Pipet 5 mL of 0.1 N sodium hydroxide into the flask, and dilute with water to volume. Adjust with either 0.1 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 6.0. [NOTE—The resulting solution contains the 1H-dimer and 2H-dimer, and the resulting solution may be cloudy.]

System suitability solution: Add 3 mL of acetonitrile to 7 mL of *System suitability stock solution* to clear the cloudy solution, and mix well.

Standard solution: 0.25 mg/mL of USP Losartan Potassium RS in *Solution A*. Pass through a PTFE or equivalent filter of 0.45- μ m pore size.

Sample stock solution: Transfer 10 Tablets to a 500-mL volumetric flask, add *Solution A* to fill the flask to about 50% of the final volume, and sonicate with intermittent shaking for 15 min. Sonicate for an additional 10 min. Dilute with *Solution A* to volume, and mix well.

Sample solution: 0.25 mg/mL of losartan potassium in *Solution A* from the *Sample stock solution*. Mix well. Pass an aliquot of the solution through a PTFE filter of 0.45- μ m pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 3.9-mm \times 15-cm; 5- μ m packing L7

Flow rate: 1.0 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for the losartan, 1H-dimer, and 2H-dimer peaks; *System suitability solution*

Resolution: NLT 2.0 between the 1H-dimer and 2H-dimer, *System suitability solution*

Column efficiency: NLT 3000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of losartan from the *Sample solution*

r_S = peak response of losartan from the *Standard solution*

C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of losartan potassium in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

DISSOLUTION <711>

Test 1

Medium: Water; 900 mL, deaerated

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: (L/1000) mg/mL of USP Losartan Potassium RS in *Medium*, where L is the Tablet label claim, in mg

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Analysis: Determine the amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved by using one of the following procedures:

Instrumental conditions

Analytical wavelength: Maximum absorbance at about 256 nm

Path length: See *Table 2* or make the appropriate dilution of the solutions with *Medium* to be within the linearity range of the spectrophotometer.

Table 2

Tablet Strength (mg/Tablet)	Cell Size (cm)
25	1.0
50	0.5
100	0.2

Blank: *Medium*

Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Chromatographic procedure

Solution A: 0.1% v/v phosphoric acid in water

Mobile phase: Acetonitrile and *Solution A* (40:60)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.0-mm × 25-cm; 5-μm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: NLT 1.5 times the retention time of losartan

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 30 min

Buffer: 1.4 g/L of anhydrous monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.3 ± 0.1 .

Mobile phase: Methanol, acetonitrile, and *Buffer* (20:20:60)

Standard solution: 0.028 mg/mL of USP Losartan Potassium RS in *Medium*

Sample solution

For Tablets labeled to contain 25 mg: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

For Tablets labeled to contain 50 and 100 mg: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size. Further dilute the filtrate with *Medium* to prepare a 0.028-mg/mL solution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 15-cm; 5-μm packing L10

Column temperature: 45°

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

Medium: Water; 900 mL, deaerated

Apparatus 2: 50 rpm

Time: 30 min for 25-mg and 50-mg Tablet strengths, and 45 min for 100-mg Tablet strength

Buffer: 0.025 M phosphoric acid. Adjust with 1 N sodium hydroxide to a pH of 2.15.

Mobile phase: Acetonitrile and *Buffer* (400:600)

Standard stock solution: 0.27 mg/mL of USP Losartan Potassium RS prepared as follows. Add methanol to USP Losartan Potassium RS to fill about 10% of the volume of the flask, and add *Medium* to fill about 50% of the volume of the flask. Sonicate for NLT 15 min. Cool to room temperature, and dilute with *Medium* to volume.

Standard solution: Prepare as directed in *Table 3* from the *Standard stock solution*.

Table 3

Tablet Strength (mg/Tablet)	Concentration (mg/mL)
25	0.027
50	0.054
100	0.108

Sample solution: Pass a portion of the solution under test through a suitable polyethylene filter of 10-μm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 3.5-μm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of losartan from the *Sample solution*

r_S = peak response of losartan from the *Standard solution*

C_S = concentration of USP Losartan Potassium RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) is dissolved for 25-mg and 50-mg Tablet strengths. NLT 80% (Q) of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) is dissolved for 100-mg Tablet strength.

• **UNIFORMITY OF DOSAGE UNITS** (905)

Procedure for content uniformity

Buffer: Dissolve 1.36 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

Diluent: Dissolve 17.42 g of dibasic potassium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 8.0. Dilute with water to a volume of 1000 mL, and mix well. Further dilute with water (1 in 10), and mix well.

Mobile phase: Acetonitrile and Buffer (60:40)

Standard solution: 0.05 mg/mL of USP Losartan Potassium RS in Diluent

Sample stock solution: Transfer 1 Tablet to a 100-mL volumetric flask, add about 65 mL of Diluent, and shake mechanically for 30 min. Dilute with Diluent to volume, and mix well.

Sample solution: 0.05 mg/mL of losartan potassium in Diluent from the Sample stock solution. Filter an aliquot of the solution, and use the filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 10-μm packing L7

Flow rate: 1.4 mL/min

Injection volume: 20 μL

System suitability

Sample: Standard solution

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClN_6O$) in the portion of the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of losartan from the Sample solution

r_S = peak response of losartan from the Standard solution

C_S = concentration of USP Losartan Potassium RS in the Standard solution (mg/mL)

C_U = nominal concentration of losartan potassium in the Sample solution (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Prepare as directed in the Assay.

Standard stock solution: Use the Standard solution, prepared as directed in the Assay.

Standard solution: 2.5 μg/mL of USP Losartan Potassium RS in Solution A from the Standard stock solution

Sensitivity solution: Dilute 1 mL of the Standard solution to 10 mL in Solution A.

System suitability

Samples: System suitability solution, Standard solution, and Sensitivity solution

Suitability requirements

Tailing factor: NMT 2.0 for the losartan, 1H-dimer, and 2H-dimer peaks; System suitability solution

Resolution: NLT 2.0 between the 1H-dimer and 2H-dimer, System suitability solution

Column efficiency: NLT 3000 theoretical plates, Standard solution

Tailing factor: NMT 2.0, Standard solution

Relative standard deviation: NMT 5.0%, Standard solution

Signal-to-noise ratio: NLT 10 for the losartan peak from the first injection. If this is not met, then the Signal-to-noise ratio must be greater than 3 with a relative standard deviation of area counts less than 25% for three replicate injections, Sensitivity solution.

Analysis

Samples: Standard solution and Sample solution

[NOTE—Identify the peaks using the relative retention times provided in Table 4.]

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the Sample solution

r_S = peak response of losartan from the Standard solution

C_S = concentration of USP Losartan Potassium RS in the Standard solution (mg/mL)

C_U = nominal concentration of losartan potassium in the Sample solution (mg/mL)

Acceptance criteria: See Table 4.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Losartan	1.0	—
1H-Dimer ^a	2.4	0.5
2H-Dimer ^b	2.9	0.5
Total impurities ^c	—	1.0

^a 5-[4'-[(2-Butyl-5-[(5-[4'-[(2-butyl-4-chloro-5-hydroxymethyl-1H-imidazol-1-yl)methyl]biphenyl-2-yl)-1H-tetrazol-1-yl)methyl]-4-chloro-1H-imidazol-1-yl)methyl]biphenyl-2-yl]tetrazol, potassium salt.

^b 5-[4'-[(2-Butyl-5-[(5-[4'-[(2-butyl-4-chloro-5-hydroxymethyl-1H-imidazol-1-yl)methyl]biphenyl-2-yl)-2H-tetrazol-2-yl)methyl]-4-chloro-1H-imidazol-1-yl)methyl]biphenyl-2-yl]tetrazol, potassium salt.

^c The total impurities include the sum of all the specified impurities and the sum of all the unspecified impurities. Disregard peaks less than 0.1%.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Store in tightly closed containers, protected from light, at controlled room temperature.

• **LABELING:** When more than one Dissolution test is given, the labeling states the test used only if Test 1 is not used.

• **USP REFERENCE STANDARDS** (11)
USP Losartan Potassium RS

Losartan Potassium and Hydrochlorothiazide Tablets

DEFINITION

Losartan Potassium and Hydrochlorothiazide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amounts of losartan potassium ($C_{22}H_{22}ClN_6O$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

• **A.** The retention times of the major peaks of the Sample solution correspond to those of the Standard solution, as obtained in the Assay.

ASSAY

• **PROCEDURE**

Buffer A: 2.76 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

Buffer B: 1.25 g/L of monobasic potassium phosphate and 1.5 g/L of dibasic sodium phosphate in water. The pH of the resulting solution is about 7.0–7.5.

Diluent: Acetonitrile and *Buffer A* (3:2)

Solution A: Acetonitrile and *Buffer B* (7:93)

Solution B: Use acetonitrile.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
12	92	8
28	38	62
30	100	0
35	100	0

Standard solution: Transfer USP Losartan Potassium RS and USP Hydrochlorothiazide RS into a suitable volumetric flask, and dissolve in *Diluent* (50% of the volume of the flask). Dilute with *Buffer A* to volume to obtain a solution having concentrations as directed in *Table 2*. Pass a portion of the solution through a PTFE or equivalent filter of 0.45- μ m pore size.

Table 2

Tablet Strength Losartan Potassium/Hydrochlorothiazide (mg)	Concentration of USP Losartan Potassium RS (mg/mL)	Concentration of USP Hydrochlorothiazide RS (mg/mL)
50/12.5	0.4	0.1
100/12.5	0.4	0.05
100/25	0.4	0.1

Sample stock solution: Transfer 10 Tablets into a suitable volumetric flask and add *Diluent* as directed in *Table 3*. Mix well and mechanically shake or stir until the solid is dispersed. Dilute with *Buffer A* to volume, and sonicate.

Table 3

Tablet Strength Losartan Potassium/Hydrochlorothiazide (mg)	Flask Size (mL)	Volume of Diluent (mL)
50/12.5	250	210
100/12.5	500	420
100/25	500	420

Sample solution: Dilute a portion of the *Sample stock solution* first with acetonitrile (20% of the volume of the flask) and then with *Buffer A*, to obtain a solution having nominal concentrations as directed in *Table 4*. Pass a portion of this solution through a PTFE or equivalent filter of 0.45- μ m pore size, and use the filtrate.

Table 4

Tablet Strength Losartan Potassium/Hydrochlorothiazide (mg)	Concentration of USP Losartan Potassium RS (mg/mL)	Concentration of USP Hydrochlorothiazide RS (mg/mL)
50/12.5	0.4	0.1
100/12.5	0.4	0.05
100/25	0.4	0.1

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm \times 15-cm; 5- μ m packing L7

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: Less than 2.5 for the losartan peak

Relative standard deviation: Less than 2.0% for both hydrochlorothiazide and losartan peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) or hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of losartan or hydrochlorothiazide from the *Sample solution*

r_S = peak response of losartan or hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Losartan Potassium RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of losartan potassium or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

Change to read:

• DISSOLUTION (711)

• **Test 1** (RB 1-jun-2016)

Medium: Water; 900 mL, deaerated

Apparatus 1: 100 rpm

Time: 30 min for both losartan and hydrochlorothiazide

Buffer: Dissolve 1.36 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Buffer* (2:3)

Losartan potassium stock solution: 0.44 mg/mL of USP Losartan Potassium RS in *Medium*

Hydrochlorothiazide stock solution: 0.14 mg/mL of USP Hydrochlorothiazide RS prepared by dissolving in methanol (10% of the volume of the flask). Dilute with *Medium* to volume.

Standard solution: Transfer the appropriate volumes of *Losartan potassium stock solution* and *Hydrochlorothiazide stock solution* to a 100-mL volumetric flask according to the dilution schemes in *Table 5*. Dilute with *Medium* to volume.

Table 5

Tablet Strength Losartan Potassium/Hydrochlorothiazide (mg)	Aliquot of Losartan Potassium Stock Solution (mL)	Aliquot of Hydrochlorothiazide Stock Solution (mL)
50/12.5	12.5	10.0
100/12.5	25.0	10.0
100/25	25.0	20.0

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 230 nm**Column:** 4.6-mm × 25-cm; 10-μm packing L7**Column temperature:** 35°**Flow rate:** 2.3 mL/min**Injection volume:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 2 between the hydrochlorothiazide and losartan peaks**Relative standard deviation:** NMT 2.0% for both the hydrochlorothiazide and losartan peaks**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of losartan potassium (C₂₂H₂₂ClKN₆O) or hydrochlorothiazide (C₇H₈ClN₃O₄S₂) dissolved:

$$\text{Result} = (r_u/r_s) \times (C_s/L) \times V \times 100$$

 r_u = peak response of losartan or hydrochlorothiazide from the *Sample solution* r_s = peak response of losartan or hydrochlorothiazide from the *Standard solution* C_s = concentration of USP Losartan Potassium RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL) L = label claim (mg/Tablet) V = volume of *Medium*, 900 mL**Tolerances:** NLT 85% (Q) of the labeled amount of losartan potassium (C₂₂H₂₂ClKN₆O) and NLT 75% (Q) of the labeled amount of hydrochlorothiazide (C₇H₈ClN₃O₄S₂) is dissolved.• **Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.**Medium, Apparatus 1, and Time:** Proceed as directed in *Test 1*.**Buffer:** Dissolve 1.78 g of dibasic sodium phosphate dihydrate in 1 L of water. Adjust with phosphoric acid to a pH of 6.5.**Mobile phase:** Acetonitrile and *Buffer* (32:68)**Diluent:** Acetonitrile and water (40:60)**Standard stock solution 1:** 1.1 mg/mL of USP Losartan Potassium RS in *Diluent*. Sonication may be necessary for complete dissolution.**Standard stock solution 2:** 0.28 mg/mL of USP Hydrochlorothiazide RS in *Diluent*. Sonication may be necessary for complete dissolution.**Standard solution:** Transfer appropriate volumes of *Standard stock solution 1* and *Standard stock solution 2* to a 100-mL volumetric flask according to the dilution schemes in *Table 6*. Dilute with *Medium* to volume.**Table 6**

Tablet Strength Losartan Potassium/ Hydrochlorothiazide (mg)	Aliquot of Standard Stock Solution 1 (mL)	Aliquot of Standard Stock Solution 2 (mL)
50/12.5	5	5
100/12.5	10	5
100/25	10	10

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 225 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Autosampler temperature:** 8°**Flow rate:** 1.2 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0% for both the hydrochlorothiazide and losartan peaks**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of losartan potassium (C₂₂H₂₂ClKN₆O) and hydrochlorothiazide (C₇H₈ClN₃O₄S₂) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times (1/L) \times V \times 100$$

 r_u = peak response of losartan or hydrochlorothiazide from the *Sample solution* r_s = peak response of losartan or hydrochlorothiazide from the *Standard solution* C_s = concentration of USP Losartan Potassium RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL) L = label claim (mg/Tablet) V = volume of *Medium*, 900 mL**Tolerances:** NLT 85% (Q) of the labeled amount of losartan potassium (C₂₂H₂₂ClKN₆O) and NLT 80% (Q) of the labeled amount of hydrochlorothiazide (C₇H₈ClN₃O₄S₂) is dissolved. (RB 1-Jun-2016)• **UNIFORMITY OF DOSAGE UNITS (905)****Procedure for content uniformity****Buffer A:** Prepare as directed in the *Assay*.**Buffer B:** Dissolve 1.36 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.**Diluent:** Prepare a mixture of acetonitrile and *Buffer A* (3:2).**Mobile phase:** Acetonitrile and *Buffer B* (2:3)**Standard stock solution 1:** 0.46 mg/mL of USP Losartan Potassium RS prepared by dissolving in *Diluent* (50% of the total volume of the flask). Mechanically shake for 15 min or until dissolved. Dilute with *Buffer A* to volume.**Standard stock solution 2:** 0.35 mg/mL of USP Hydrochlorothiazide RS prepared by dissolving in *Diluent* (50% of the total volume of the flask). Mechanically shake for 15 min or until dissolved. Dilute with *Buffer A* to volume.**Standard solution:** Transfer aliquots of *Standard stock solution 1* and *Standard stock solution 2* into a suitable volumetric flask, and add *Diluent*, up to 42% of the total volume of the flask. Dilute with *Buffer A* to volume, mix well, and sonicate for 2 min to obtain a solution having concentrations based on Tablet strength as listed in *Table 7*. Pass a portion of the solution through a PTFE or equivalent filter of 0.45-μm pore size, and use the filtrate.**Table 7**

Tablet Strength Losartan Potassium/ Hydrochlorothiazide (mg)	Concentration of USP Losartan Potassium RS (mg/mL)	Concentration of USP Hydrochloro- thiazide RS (mg/mL)
50/12.5	0.06	0.014
100/12.5	0.06	0.007
100/25	0.06	0.014

Sample stock solution: Transfer 1 Tablet into a suitable volumetric flask, and add *Diluent* as directed in *Table 7*.

ble 8. Mix well, and mechanically shake for 30 min or until the solid is finely dispersed. Dilute with *Buffer A* to volume, and mix well.

Table 8

Tablet Strength Losartan Potassium/ Hydrochlorothiazide (mg)	Flask Size (mL)	Volume of Diluent (mL)
50/12.5	100	50
100/12.5	200	100
100/25	200	100

Sample solution: Dilute 10 mL of the *Sample stock solution* in a 100-mL volumetric flask, with 45 mL of *Diluent*, and then dilute with *Buffer A* to volume. Pass an aliquot of the solution through a PTFE or equivalent filter of 0.45- μ m pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 25-cm; 10- μ m packing L7

Column temperature: 35°

Flow rate: 2.3 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2 between the hydrochlorothiazide and losartan peaks

Relative standard deviation: NMT 2.0% for both the hydrochlorothiazide and losartan peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$) or hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of losartan or hydrochlorothiazide from the *Sample solution*

r_S = peak response of losartan or hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Losartan Potassium RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of losartan potassium or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Buffer A, Buffer B, Diluent, Solution A, Solution B, Mobile phase, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Chlorothiazide standard solution: 0.1 mg/mL of USP Chlorothiazide RS prepared by dissolving in *Diluent* (50% of the volume of the flask). Dilute with *Buffer A* to volume, and sonicate.

Benzothiadiazine related compound A standard solution: 0.1 mg/mL of USP Benzothiadiazine Related Compound A RS prepared by dissolving in *Diluent* (50% of the volume of the flask). Dilute with *Buffer A* to volume, and sonicate.

Stressed losartan solution: [NOTE—This solution contains the degradates 1-*H*-dimer and 2-*H*-dimer and losartan potassium.] Weigh 12 mg of the USP Losartan Potassium RS in a 50-mL flask. Dissolve in 5 mL of water. Pipet 5.0 mL of 0.1 N hydrochloric acid into this

solution, and place it in an oven at 105° for 1–2 h. Remove from the oven and allow to cool to room temperature. Pipet 5.0 mL of 0.1 N sodium hydroxide into the flask, and dilute with water to volume.

Diluted standard solution: Dilute portions of the *Standard solution* and *Benzothiadiazine related compound A standard solution* first with acetonitrile (30% of the volume of the flask), then with *Buffer A* to obtain a solution having nominal concentrations based on Tablet strength as listed in Table 9.

Table 9

Tablet Strength Losartan Potassium/ Hydrochlorothiazide (mg)	Concentration of USP Losartan Potassium RS (μ g/mL)	Concentration of USP Hydrochlorothiazide RS (μ g/mL)	Concentration of USP Benzothiadiazine Related Compound A RS (μ g/mL)
50/12.5	4	1	1
100/12.5	4	0.5	1
100/25	4	1	1

System suitability solution: Dissolve weighed quantities of USP Losartan Potassium RS and USP Hydrochlorothiazide RS in a suitable volumetric flask in *Diluent* (50% of the volume of the flask). Add the *Stressed losartan solution*, about 25% of the volume of the flask, into the same flask. Transfer appropriate amounts of *Chlorothiazide standard solution* and *Benzothiadiazine related compound A standard solution* into the same flask, and dilute with *Buffer A* to volume to obtain a solution having a known concentration of about 0.4 mg/mL of losartan, 0.1 mg/mL of hydrochlorothiazide, and 0.001 mg/mL each of benzothiadiazine related compound A and chlorothiazide. Adjust with phosphoric acid to a pH of 2.5, and mix well. Pass an aliquot of the solution through a PTFE or equivalent filter of 0.45- μ m pore size, and use the filtrate.

Limit of quantitation solution: Pipet 5.0 mL of the *Diluted standard solution* into a 50-mL volumetric flask. Add 15 mL of acetonitrile, dilute with *Buffer A* to volume, and mix well.

System suitability

Samples: *Standard solution*, *Diluted standard solution*, *System suitability solution*, and *Limit of quantitation solution*

Suitability requirements

Resolution: Greater than 1.5 between chlorothiazide and benzothiadiazine related compound A; greater than 1.5 between the benzothiadiazine related compound A and hydrochlorothiazide peak, *System suitability solution*

Tailing factor: Less than 2.5 for the losartan peak, *Standard solution*

Relative standard deviation: Less than 2.0% for both the hydrochlorothiazide and losartan peaks, *Standard solution*; less than 10.0% for both the hydrochlorothiazide and losartan peaks, *Diluted standard solution*

Signal-to-noise ratio: NLT 10 for each component from the first injection. If this is not met, then the signal-to-noise ratio must be greater than 3 with a relative standard deviation of area counts less than 25% for three replicate injections, *Limit of quantitation solution*

Analysis

Samples: *Sample solution* and *Diluted standard solution* [NOTE—The run time is about 1.6 times the retention time of the losartan peak. Identify the peaks using the relative retention times provided in Table 10.]

Calculate the percentage of benzothiadiazine related compound A (expressed as hydrochlorothiazide equivalent) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response of benzothiadiazine related compound A from the *Sample solution*
 r_S = peak response of benzothiadiazine related compound A from the *Diluted standard solution*
 C_S = concentration of USP Benzothiadiazine Related Compound A RS in the *Diluted standard solution* (mg/mL)
 C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of hydrochlorothiazide, 298
 M_{r2} = molecular weight of benzothiadiazine related compound A, 286

Calculate the percentage of each specified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of each individual impurity from the *Sample solution*
 r_S = peak response of losartan from the *Diluted standard solution*
 C_S = concentration of USP Losartan Potassium RS in the *Diluted standard solution* (mg/mL)
 C_U = nominal concentration of losartan potassium in the *Sample solution* (mg/mL)

For Tablet strengths of 50/12.5 and 100/25 for losartan potassium/hydrochlorothiazide, respectively, calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of each individual impurity from the *Sample solution*
 r_S = peak response of losartan from the *Diluted standard solution*
 C_S = concentration of USP Losartan Potassium RS in the *Diluted standard solution* (mg/mL)
 C_U = nominal concentration of losartan potassium in the *Sample solution* (mg/mL)

For a Tablet strength of 100/12.5 for losartan potassium/hydrochlorothiazide, calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of each individual impurity from the *Sample solution*
 r_S = peak response of hydrochlorothiazide from the *Diluted standard solution*
 C_S = concentration of USP Hydrochlorothiazide RS in the *Diluted standard solution* (mg/mL)
 C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria See Table 10.

Table 10

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorothiazide ^a	0.57	—
Benzothiadiazine related compound A	0.69	1.0
Hydrochlorothiazide	1.0	—
Losartan	2.7	—
1-H-Dimer ^b	3.3	0.5
2-H-Dimer ^c	3.5	0.5
Total impurities ^d	—	2.0

^a This process impurity (not a degradation product) is related to hydrochlorothiazide and is controlled in the drug substance.

^b Related to losartan potassium: 5-[4'-[(2-butyl-5-[(2-butyl-4-chloro-5-hydroxymethyl-1H-imidazol-1-yl)methyl]biphenyl-2-yl)-1H-tetrazol-1-yl]methyl]-4-chloro-1H-imidazol-1-yl)methyl]biphenyl-2-yl]tetrazol, potassium salt.

^c Related to losartan potassium: 5-[4'-[(2-butyl-5-[(2-butyl-4-chloro-5-hydroxymethyl-1H-imidazol-1-yl)methyl]biphenyl-2-yl)-2H-tetrazol-2-yl]methyl]-4-chloro-1H-imidazol-1-yl)methyl]biphenyl-2-yl]tetrazol, potassium salt.

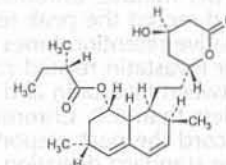
^d Total impurities include the sum of all the specified impurities and the unspecified impurities that are equal to or greater than 0.1%.

ADDITIONAL REQUIREMENTS

Add the following:

- LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
 • (RB 1-Jun-2016)
- PACKAGING AND STORAGE:** Preserve in tightly closed containers protected from light, and store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
 USP Benzothiadiazine Related Compound A RS
 4-Amino-6-chloro-1,3-benzenedisulfonamide.
 $C_{10}H_8ClN_3O_4S_2$ 285.73
 USP Chlorothiazide RS
 USP Hydrochlorothiazide RS
 USP Losartan Potassium RS

Lovastatin



- $C_{24}H_{36}O_5$ 404.54
 Butanoic acid, 2-methyl-, 1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-naphthalenyl ester, [1S-[1 α (R*),3 α ,7 β ,8 β (2S*,4S*),8 α]]-
 (5)-2-Methylbutyric acid, 8-ester with (4R,6R)-6-[2-[(1S,2S,6R,8S,8aR)-1,2,6,7,8,8a-hexahydro-8-hydroxy-2,6-dimethyl-1-naphthyl]ethyl]tetrahydro-4-hydroxy-2H-pyran-2-one [75330-75-5].

» Lovastatin contains not less than 98.5 percent and not more than 101.0 percent of $C_{24}H_{36}O_5$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers under nitrogen in a cold place.

USP Reference standards (11)—

USP Lovastatin RS

USP Lovastatin Related Compound A RS

[Dihydro-lovastatin][butanoic acid, 2-methyl-, 1,2,3,4,4a,7,8,8a-octahydro-3,7-dimethyl-8-[2(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl]-1-naphthalenyl ester, [1S-[1 α (R*),3 α ,7 β ,8 β (2S*,4S*),-8 α]]]-C₂₄H₃₈O₅ 406.56

Identification—

A: Infrared Absorption (197M).

B: Ultraviolet Absorption (197U)—

Solution: 10 μ g per mL.

Medium: acetonitrile.

Specific rotation (781S): between +324° and +338°.

Test solution: 5 mg per mL, in acetonitrile.

Loss on drying (731)—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 0.3% of its weight.**Residue on ignition** (281): not more than 0.2%.**Delete the following:**

*Heavy metals, Method II (231): 0.002%. (Official 1-Jan-2018)

Limit of lovastatin related compound A—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and 0.01 M phosphoric acid (13:7). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability solution—Dissolve accurately weighed quantities of USP Lovastatin RS and USP Lovastatin Related Compound A RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, to obtain a solution containing 2.0 μ g of each per mL.

Standard solution—Dissolve an accurately weighed quantity of USP Lovastatin RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 2.0 μ g per mL.

Test solution—Transfer about 25 mg of Lovastatin, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 200-nm detector and a 4.6-mm \times 25-cm column that contains 5- μ m packing L7. The column temperature is maintained at 40°. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for lovastatin and 1.3 for lovastatin related compound A; and the resolution, R , between lovastatin and lovastatin related compound A is not less than 6.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all the peak responses. Calculate the percentage of lovastatin related compound A in the portion of Lovastatin taken by the formula:

$$2.5F(C/W)(r_U/r_S)$$

in which F is the response factor for lovastatin related compound A and is equal to 1.6; C is the concentration, in μ g per mL, of USP Lovastatin RS in the *Standard solution*; W is the weight, in mg, of Lovastatin in the *Test solution*; r_U is the peak response for lovastatin related compound A obtained from the *Test solution*; and r_S is the peak response for lovastatin obtained from the *Standard solution*: not more than 0.5% of lovastatin related compound A is found.

Chromatographic purity—

Solution A—Prepare a 0.001 M phosphoric acid solution, adjusted with 1 M sodium hydroxide to a pH of 4.0.

Solution B—Use acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability solution—Dissolve accurately weighed quantities of USP Lovastatin RS and compactin in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution containing 2.0 μ g of each per mL.

Standard solution—Dissolve an accurately weighed quantity of USP Lovastatin RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 2.0 μ g per mL.

Test solution—Transfer about 25 mg of Lovastatin, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 238-nm detector and a 4.0-mm \times 12.5-cm column that contains 4- μ m packing L1. The column temperature is maintained at 40°. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–2	60	40	isocratic
2–5	60→45	40→55	linear gradient
5–8	45	55	isocratic
8–16	45→10	55→90	linear gradient
16–25	10	90	isocratic
25–27	10→60	90→40	linear gradient
27–35	60	40	isocratic

Chromatograph the *System suitability solution* and the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.00 for lovastatin and 0.85 for compactin; the resolution, R , between lovastatin and compactin is not less than 3.5; and the relative standard deviation for replicate injections is not more than 5%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all the peak responses. Calculate the percentage of each impurity in the portion of Lovastatin taken by the formula:

$$2.5(C/W)(r_i/r_S)F$$

in which C is the concentration, in μ g per mL, of USP Lovastatin RS in the *Standard solution*; W is the weight, in mg, of Lovastatin in the *Test solution*; r_i is the peak response for each impurity obtained from the *Test solution*; r_S is the peak response for lovastatin obtained from the *Standard solution*; and F is the response factor for each impurity and is equal to 1.4 for the impurity with a relative retention time of about 0.73 and 1.0 for all other impurities. Disregard any peak with less than 0.04%: not more than 0.2% of any individual impurity is found; and not more than 1.0% of total impurities is found.

Assay—

Dilute phosphoric acid—Transfer 1 mL of phosphoric acid to a 1-L volumetric flask, and dilute with water to volume.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *Dilute phosphoric acid* (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Lovastatin RS in acetonitrile to obtain a solution having a known concentration of about 0.3 mg per mL.

Assay preparation—Transfer about 30 mg of Lovastatin, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.

Chromatographic system (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 238-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 1.4, and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{24}H_{36}O_5$ in the portion of Lovastatin taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Lovastatin RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Lovastatin Tablets

» Lovastatin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lovastatin ($C_{24}H_{36}O_5$).

Packaging and storage—Preserve in well-closed, light-resistant containers. Protect from light and store either in a cool place or at controlled room temperature.

USP Reference standards <11>—

USP Lovastatin RS

Identification—

A: Thin-Layer Chromatographic Identification Test <201>—*Test solution*:

FOR TABLETS CONTAINING 10 MG OF LOVASTATIN—Transfer 1 Tablet to a centrifuge tube, add 0.4 mL of water and 1.6 mL of acetonitrile. Mix on a vortex mixer until the tablet disintegrates, and sonicate for 4 minutes. Centrifuge for 4 minutes, and use the clear supernatant.

FOR TABLETS CONTAINING 20 MG OR 40 MG OF LOVASTATIN—Transfer 1 Tablet to a centrifuge tube, add 1 mL of water and 4.0 mL of acetonitrile. Mix on a vortex mixer until the tablet disintegrates, and sonicate for 4 minutes. Centrifuge for 4 minutes, and use the clear supernatant.

Standard solution—Prepare a solution of USP Lovastatin RS in acetonitrile containing 8 mg per mL.

Application volume: 5 μL of the *Test solution*, 3 μL of the *Standard solution* for 10-mg and 20-mg Tablets, 5 μL of the *Standard solution* for 40-mg Tablets.

Developing solvent solution: a mixture of cyclohexane, chloroform, and isopropyl alcohol (5:2:1).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution <711>—

Medium—Dissolve 1.38 g of monobasic sodium phosphate and 20 g of sodium lauryl sulfate in 900 mL of water. Adjust with 1 N sodium hydroxide to a pH of 7.0, dilute with water to 1000 mL, and mix; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Mobile phase—Proceed as directed in the *Assay*.

Standard solution—Accurately weigh approximately 44 mg of USP Lovastatin RS into a 500-mL volumetric flask, and dissolve in no more than 20 mL of methanol. Dilute with *Medium* to volume, and mix well. Further dilute this solution with *Medium* to obtain a solution containing $L/900$ mg per mL, with L being the Tablet label claim, in mg.

Test solution—Pass the solution under test through a suitable 0.45-μm filter.

Chromatographic system (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 5-cm column that contains 5-μm packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the capacity factor, k' , is greater than 2.0; the tailing factor is less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of lovastatin dissolved by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times L}$$

in which r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively; C_S is the concentration, in mg per mL, of the *Standard solution*; 900 is the volume, in mL, of *Medium*; and L is the Tablet label claim, in mg.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{24}H_{36}O_5$ is dissolved in 30 minutes.

Uniformity of dosage units <905>: meet the requirements.

Assay—

Buffer solution—Dissolve 3.45 g of monobasic sodium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 4.0, dilute with water to 1000 mL, and mix.

Dissolving solvent—Add 3.0 mL of glacial acetic acid to 900 mL of water contained in a 1 L beaker, adjust to a pH of 4.0, determined electrometrically, by the addition of a solution of sodium hydroxide (20%), and mix. Transfer the contents of the beaker to a 1000-mL volumetric flask, dilute with water to volume, and mix. Prepare a mixture of acetonitrile and the resultant solution (80:20).

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, *Buffer solution*, and methanol (5:3:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

Standard preparation—Dissolve an accurately weighed quantity of USP Lovastatin RS in *Dissolving solvent* to obtain a solution having a known concentration of about 40 μg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of lovastatin, to a 200-mL volumetric flask. Add about 150 mL of *Dissolving solvent*, and sonicate for 20 minutes. Cool to room temperature, and allow the solution to stand for 30 minutes. Dilute with *Dissolving solvent* to volume, and mix. Centrifuge a

portion of this solution, transfer 5.0 mL of the clear supernatant to a 25-mL volumetric flask, dilute with *Dissolving solvent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains packing L1 and is maintained at 45°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is greater than 3000 theoretical plates, the tailing factor is less than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of the labeled amount of C₂₄H₃₆O₅ in the portion of Tablets taken by the formula:

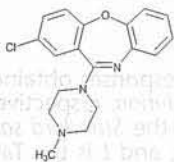
$$100(C_s / C_u)(r_u / r_s)$$

in which C_s is the concentration, in µg per mL, of USP Lovastatin RS in the *Standard preparation*; C_u is the concentration of lovastatin, in µg per mL, in the *Assay preparation*, based on the label claim; and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Loxapine Succinate

C₁₈H₁₈ClN₃O · C₄H₆O₄

445.90



Butanedioic acid, compd. with 2-chloro-11-(4-methyl-1-piperazinyl)dibenz[b,f][1,4]oxazepine (1:1); 2-Chloro-11-(4-methyl-1-piperazinyl)dibenz[b,f][1,4]oxazepine succinate (1:1) [27833-64-3].

DEFINITION

Loxapine Succinate contains NLT 98.5% and NMT 101.0% of C₁₈H₁₈ClN₃O · C₄H₆O₄, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)

Sample solution: 20 µg/mL in 0.01 N hydrochloric acid

ASSAY

• PROCEDURE

Sample: 400 mg of Loxapine Succinate

Analysis: Dissolve in 80 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)).

Calculate the percentage of C₁₈H₁₈ClN₃O · C₄H₆O₄ in the portion of Loxapine Succinate taken:

$$\text{Result} = [(V - B) \times N \times F \times 100] / W$$

V = sample titrant volume (mL)

B = blank titrant volume (mL)

N = normality of titrant (mEq/mL)

F = equivalence factor, 22.29 mg/mEq

W = sample weight (mg)

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-

Jan-2018)

Organic Impurities

• PROCEDURE

Buffer: 3.9 g/L of ammonium acetate in water. Adjust with 20% acetic acid in water or 6 N ammonium hydroxide to a pH of 7.3.

Mobile phase: Acetonitrile and Buffer (3:7)

Diluent: Acetonitrile and Buffer (7:3)

Standard solution: 10 µg/mL of USP Loxapine Succinate RS and 1.5 µg/mL each of USP Amoxapine RS and USP Loxapine Related Compound A RS in Diluent.

[NOTE—Amoxapine has been included in the *Standard solution* for peak identification purposes only.]

Sample solution: 1 mg/mL of Loxapine Succinate in Diluent

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 5-cm; 2.7-µm packing L1

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection size: 10 µL

Run time: 2 times the retention time of loxapine succinate

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between loxapine succinate and loxapine related compound A

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Loxapine Succinate taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times (1 / F) \times 100$$

r_u = peak response of each impurity from the *Sample solution*

r_s = peak response of loxapine succinate from the *Standard solution*

C_s = concentration of USP Loxapine Succinate RS in the *Standard solution* (mg/mL)

C_u = concentration of Loxapine Succinate in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxapine ^a	0.19	1.3	0.15
Chlorodibenzoxazepine ^b	0.45	0.70	0.15
Loxapine succinate	1.0	—	—

^a 2-Chloro-11-(piperazin-1-yl)dibenzo[b,f][1,4]oxazepine.

^b 2-Chlorodibenzo[b,f]-1,4-oxazepin-11-one.

^c 3-Chloro-11-(4-methylpiperazin-1-yl)dibenzo[b,f][1,4]oxazepine.

Impurity Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Loxapine related compound A ^c	1.2	1.2	0.15
Any unspecified individual impurity	—	1.0	0.10

^a 2-Chloro-11-(piperazin-1-yl)dibenzo[*b,f*][1,4]oxazepine.^b 2-Chlorodibenzo[*b,f*]-1,4-oxazepin-11-one.^c 3-Chloro-11-(4-methylpiperazin-1-yl)dibenzo[*b,f*][1,4]oxazepine.**SPECIFIC TESTS**

- **LOSS ON DRYING (731):** Dry a sample at 105° for 4 h; it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
 - USP Amoxapine RS
 - USP Loxapine Related Compound A RS
 - 3-Chloro-11-(4-methylpiperazin-1-yl)dibenzo[*b,f*][1,4]oxazepine.
 - C₁₈H₁₈ClN₃O 327.81
 - USP Loxapine Succinate RS

Loxapine Capsules**DEFINITION**

Loxapine Capsules contain an amount of loxapine succinate (C₁₈H₁₈ClN₃O · C₄H₆O₄) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of loxapine (C₁₈H₁₈ClN₃O).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Mobile phase: Dissolve 4 g of tetramethylammonium chloride in 800 mL of water, and add 200 mL of acetonitrile and 1 mL of phosphoric acid.

Standard solution: 0.136 mg/mL of USP Loxapine Succinate RS prepared as follows. Dissolve a suitable quantity of USP Loxapine Succinate RS in 0.01 N hydrochloric acid in a suitable volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.1 mg/mL of loxapine prepared as follows. Weigh the contents of NLT 20 Capsules. Transfer a portion of the contents, nominally equivalent to NLT 50 mg of loxapine, to a suitable volumetric flask. Add 10% of the flask volume of 0.1 N hydrochloric acid. Shake, and sonicate for 5 min. Dilute with *Mobile phase* to volume, and filter. Discard the first 8 mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-μm packing L10

Flow rate: 1.6 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of loxapine (C₁₈H₁₈ClN₃O) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Loxapine Succinate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of loxapine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of loxapine, 327.81

M_{r2} = molecular weight of loxapine succinate, 445.90

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS**• DISSOLUTION (711)**

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: USP Loxapine Succinate RS at a known concentration in *Medium*

Sample solution: Dilute with water as needed.

Instrumental conditions

Mode: UV

Analytical wavelength: 254 nm

Analysis

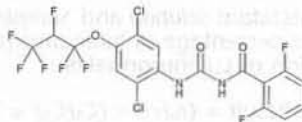
Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 75% (Q) of the labeled amount of loxapine (C₁₈H₁₈ClN₃O) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
 - USP Loxapine Succinate RS

Lufenuron

C₁₇H₈Cl₂F₈N₂O₃

511.15

Benzamide, *N*-[[[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]amino]carbonyl]-2,6-difluoro-;

1-[2,5-Dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-3-(2,6-difluorobenzoyl)urea [103055-07-8].

DEFINITION

Lufenuron contains NLT 98.0% and NMT 102.0% of lufenuron (C₁₇H₈Cl₂F₈N₂O₃), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: 0.1 mL of phosphoric acid diluted with water to 1 L

Solution B: Acetonitrile

Mobile phase: See *Table 1*. Return to original conditions, and equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	30	70
5	30	70
15	10	90
17	10	90

Diluent: Acetonitrile and water (70:30)

System suitability solution: 0.4 mg/mL of USP Lufenuron RS and 0.14 mg/mL of USP Lufenuron Related Compound G RS in *Diluent*. Sonicate if necessary to facilitate dissolution.

Standard stock solution: 0.4 mg/mL of USP Lufenuron RS in *Diluent*. Sonicate if necessary to facilitate dissolution.

Standard solution: 0.04 mg/mL of USP Lufenuron RS in *Diluent* from *Standard stock solution*

Sample stock solution: 0.4 mg/mL of Lufenuron in *Diluent*. Sonicate if necessary to facilitate dissolution.

Sample solution: 0.04 mg/mL of Lufenuron in *Diluent* from *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 255 nm

Column: 4-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for lufenuron related compound G and lufenuron are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between lufenuron related compound G and lufenuron, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lufenuron ($C_{17}H_8Cl_2F_8N_2O_3$) in the portion of Lufenuron taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Lufenuron RS in the *Standard solution* (mg/mL)
 C_U = concentration of Lufenuron in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Identification solution: 1.2 μg/mL of USP Lufenuron Related Compound B RS and 1.6 μg/mL of USP Lufenuron Related Compound C RS in *Diluent*

Diluted standard solution: 0.4 μg/mL of USP Lufenuron RS in *Diluent* from *Standard solution*

Sample solution: 0.4 mg/mL of Lufenuron in *Diluent*. Sonicate if necessary to facilitate dissolution.

Analysis

Samples: *Identification solution*, *Diluted standard solution*, and *Sample solution*

Chromatograph the *Identification solution*, and identify the components on the basis of their relative retention times, given in *Table 2*.

Calculate the percentage of each impurity in the portion of Lufenuron taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- r_U = peak response of each impurity from the *Sample solution*
 r_S = peak response of lufenuron from the *Diluted standard solution*
 C_S = concentration of USP Lufenuron RS in the *Diluted standard solution* (μg/mL)
 C_U = concentration of Lufenuron in the *Sample solution* (μg/mL)
 F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting level for impurities is 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lufenuron related compound B	0.3	0.77	0.3
Lufenuron related compound C	0.7	0.77	0.4
Lufenuron	1.0	—	—
Any other individual impurity	—	1.0	0.20
Total impurities	—	—	1.0

SPECIFIC TESTS• **LOSS ON DRYING** (731)

Analysis: Dry at 105° to constant weight.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** (11)
 USP Lufenuron RS
 USP Lufenuron Related Compound B RS
 N-[(2,5-Dichloro-4-hydroxyphenyl)carbamoyl]-2,6-difluorobenzamide.
 $C_{14}H_8Cl_2F_2N_2O_3$ 361.13

USP Lufenuron Related Compound C RS

N-[3-Chloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl-carbamoyl]-2,6-difluorobenzamide.

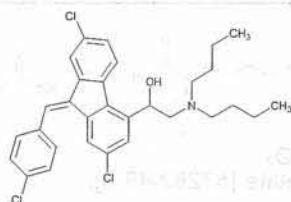
$C_{17}H_9ClF_8N_2O_3$ 476.71

USP Lufenuron Related Compound G RS

2,5-Dichloro-4-[3-(2,6-difluorobenzoyl)ureido]phenyl phenyl carbonate.

$C_{21}H_{12}Cl_2F_2N_2O_5$ 481.23

Lumefantrine



$C_{30}H_{32}Cl_3NO$ 528.94
(±)-2,7-Dichloro-9-[(*Z*)-*p*-chlorobenzylidene]-α[(dibutylamino)methyl]-fluorene-4-methanol [82186-77-4].

DEFINITION

Lumefantrine contains NLT 98.0% and NMT 102.0% of lumefantrine ($C_{30}H_{32}Cl_3NO$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the lumefantrine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer: Dissolve 5.65 g of sodium 1-hexanesulfonate and 2.75 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 2.3 before dilution with water to a final volume of 1000 mL.

Solution A: Acetonitrile and *Buffer* (300:700)

Solution B: Acetonitrile and 2-propanol (540:460)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	65	35
1.2	65	35
6.0	50	50
6.4	30	70
10.0	25	75
15.0	10	90
15.1	65	35
20.0	65	35

System suitability stock solution: 10 µg/mL of USP Lumefantrine Related Compound A RS prepared as follows. Transfer a suitable quantity of USP Lumefantrine Related Compound A RS to a volumetric flask, dissolve in 10% volume dichloromethane, and dilute with acetonitrile to volume.

System suitability solution: 1 mg/mL of USP Lumefantrine RS and 1 µg/mL of USP Lumefantrine Related Compound A RS prepared as follows. Transfer 10 mg of USP Lumefantrine RS to a 10-mL volumetric flask, and dissolve in 1 mL of dichloromethane. Add 1.0 mL of the

System suitability stock solution, and dilute with acetonitrile to volume.

Standard solution: 1 mg/mL of USP Lumefantrine RS prepared as follows. Transfer a suitable quantity of USP Lumefantrine RS to a volumetric flask, dissolve in 10% volume of dichloromethane, and dilute with acetonitrile to volume.

Sample solution: 1 mg/mL of Lumefantrine prepared as follows. Transfer a suitable quantity of Lumefantrine to a volumetric flask, dissolve in 10% volume of dichloromethane, and dilute with acetonitrile to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 50-mm; 1.8-µm packing L1

Column temperature: Beginning of column, 50°; end of column, 35°

Flow rate: 2.5 mL/min

Injection volume: 2.5 µL

Run time: 20 min

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for lumefantrine related compound A and lumefantrine are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.3 between lumefantrine and lumefantrine related compound A, *System suitability solution*

Tailing factor: NMT 2.1, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of lumefantrine ($C_{30}H_{32}Cl_3NO$) in the portion of Lumefantrine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)
Acceptance criteria: 98.0%–102.0%

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1-Jan-2018)

• ORGANIC IMPURITIES

Buffer, Solution A, Solution B, Mobile phase, System suitability stock solution, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard stock solution: 50 µg/mL of USP Lumefantrine RS prepared as follows. Transfer a suitable quantity of USP Lumefantrine RS into a volumetric flask, dissolve in 10% volume of dichloromethane, and dilute with acetonitrile to volume.

Standard solution: 1 µg/mL of USP Lumefantrine RS in acetonitrile from the *Standard stock solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of desbutyl lumefantrine or any other impurity in the portion of Lumefantrine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- r_U = peak response of desbutyl lumefantrine or any other impurity from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)
 C_U = concentration of the *Sample solution* (mg/mL)
 F = relative response factor

Acceptance criteria: See Table 2. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desbutyl lumefantrine ^a	0.68	1.1	0.05
Lumefantrine	1.0	—	—
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.3

^a (Z)-2-(Butylamino)-1-(2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl)ethanol.

SPECIFIC TESTS

• CLARITY OF SOLUTION

Hydrazine sulfate solution: Transfer 1.0 g of hydrazine sulfate to a 100-mL volumetric flask, and dissolve in and dilute with water to volume. Allow to stand for 4–6 h before use.

Methenamine solution: Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25 mL of water, insert the glass stopper, and mix to dissolve.

Primary opalescent suspension: Transfer 25.0 mL of *Hydrazine sulfate solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Stock opalescence suspension: Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. This suspension should not be used beyond 24 h after preparation.

Sample solution: Dissolve 1.0 g of Lumefantrine in dichloromethane, and dilute with dichloromethane to 10.0 mL.

Standard suspension: Transfer 5.0 mL of *Stock opalescence suspension* to a 100-mL volumetric flask, and dilute with water to volume. Prepare only if the *Sample solution* is not as clear as water or dichloromethane.

Analysis

Samples: *Sample solution*, *Standard suspension*, water, and dichloromethane

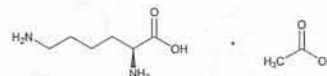
Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension* and dichloromethane to separate matching test tubes. Compare the *Sample solution*, *Standard suspension*, water, and dichloromethane in diffused daylight, viewing vertically against a black background. The diffusion of light must be such that the *Standard suspension* can readily be distinguished from dichloromethane. If the *Sample solution* is as clear as water or dichloromethane, it is not necessary to prepare the *Standard suspension*.

Acceptance criteria: The *Sample solution* shows the same or more clarity than water, dichloromethane, or the *Standard suspension*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **USP REFERENCE STANDARDS (11)**
 USP Lumefantrine RS
 USP Lumefantrine Related Compound A RS
 (RS,Z)-2-(Dibutylamino)-2-(2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl)ethanol.
 $C_{30}H_{32}Cl_3NO$ 528.94

Lysine Acetate



$C_6H_{14}N_2O_2 \cdot C_2H_4O_2$
 L-Lysine monoacetate [57282-49-2].

206.24

DEFINITION

Lysine Acetate contains NLT 98.0% and NMT 102.0% of L-lysine acetate ($C_6H_{14}N_2O_2 \cdot C_2H_4O_2$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

ASSAY

• PROCEDURE

Sample: 100 mg of Lysine Acetate

Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrimetric system
 (See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with the *Titrant*. Perform the *Blank* determination.

Calculate the percentage of lysine acetate ($C_6H_{14}N_2O_2 \cdot C_2H_4O_2$) in the *Sample* taken:

$$\text{Result} = \left[\frac{(V_S - V_B) \times N \times F}{W} \right] \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 103.1 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.4%
- **CHLORIDE AND SULFATE, Chloride (221)**
 Standard solution: 0.50 mL of 0.020 N hydrochloric acid
 Sample: 0.73 g of Lysine Acetate
 Acceptance criteria: NMT 0.05%
- **CHLORIDE AND SULFATE, Sulfate (221)**
 Standard solution: 0.10 mL of 0.020 N sulfuric acid
 Sample: 0.33 g of Lysine Acetate
 Acceptance criteria: NMT 0.03%
- **IRON (241):** NMT 30 ppm

Delete the following:

- **HEAVY METALS, Method I (231):** NMT 15 ppm (Official 1-Jan-2018)

• **RELATED COMPOUNDS**

Standard solution: 0.05 mg/mL of USP L-Lysine Acetate RS in water

[NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]

Sample solution: 10 mg/mL of Lysine Acetate in water

System suitability solution: 0.4 mg/mL each of USP L-Lysine Acetate RS and USP Arginine Hydrochloride RS

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL

Developing solvent system: Isopropyl alcohol and ammonium hydroxide (7:3)

Spray reagent: 0.2 g of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

System suitability

Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

Analysis

Samples: *Standard solution*, *System suitability solution*, and *Sample solution*

Dry the plate between 100° and 105° until the ammonia completely disappears. Spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.

Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

• **OPTICAL ROTATION, Specific Rotation (781S)**

Sample solution: 100 mg/mL in water

Acceptance criteria: +8.4° to +9.9°

• **LOSS ON DRYING (731):** Dry a sample at 80° for 3 h: it loses NMT 0.2% of its weight.

ADDITIONAL REQUIREMENTS

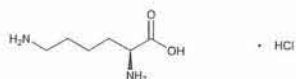
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**

USP Arginine Hydrochloride RS

USP L-Lysine Acetate RS

Lysine Hydrochloride



$C_6H_{14}N_2O_2 \cdot HCl$

L-Lysine hydrochloride [657-27-2].

182.65

DEFINITION

Lysine Hydrochloride contains NLT 98.5% and NMT 101.5% of L-lysine hydrochloride ($C_6H_{14}N_2O_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION (197K)**

ASSAY

• **PROCEDURE**

Sample: 90 mg of Lysine Hydrochloride

Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS, and titrate with the *Titrant*. Perform the *Blank* determination.

Calculate the percentage of lysine hydrochloride

($C_6H_{14}N_2O_2 \cdot HCl$) in the *Sample* taken:

$$\text{Result} = \left\{ \frac{(V_S - V_B) \times N \times F}{W} \right\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 91.33 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

OTHER COMPONENTS

• **CONTENT OF CHLORIDE**

Sample: 350 mg of Lysine Hydrochloride

Blank: 140 mL of water

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a porcelain casserole, and add 140 mL of water and 1 mL of dichlorofluorescein TS. Titrate with the *Titrant* until the silver chloride flocculates and the mixture acquires a faint pink color. Perform the *Blank* determination.

Calculate the percentage of chloride (Cl) in the *Sample* taken:

$$\text{Result} = \left\{ \frac{(V_S - V_B) \times N \times F}{W} \right\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 35.45 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 19.0%–19.6%

IMPURITIES

• **RESIDUE ON IGNITION (281):** NMT 0.1%

• **CHLORIDE AND SULFATE, Sulfate (221)**

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.33 g of Lysine Hydrochloride

Acceptance criteria: NMT 0.03%

• **IRON (241):** NMT 30 ppm

Delete the following:

• **HEAVY METALS, Method I (231):** NMT 15 ppm • (Official 1-

Jan-2018)

• **RELATED COMPOUNDS**

Standard solution: 0.05 mg/mL of USP L-Lysine Hydrochloride RS in water. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]

Sample solution: 10 mg/mL of Lysine Hydrochloride in water

System suitability solution: 0.4 mg/mL each of USP L-Lysine Hydrochloride RS and USP Arginine Hydrochloride RS

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Developing solvent system: Isopropyl alcohol and ammonium hydroxide (7:3)

Spray reagent: 0.2 g of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

System suitability

Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

Analysis

Samples: *Standard solution*, *System suitability solution*, and *Sample solution*

Dry the plate between 100° and 105° until the ammonia completely disappears. Spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.

Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

• OPTICAL ROTATION, *Specific Rotation* (781S)

Sample solution: 80 mg/mL in 6 N hydrochloric acid

Acceptance criteria: +20.4° to +21.4°

• LOSS ON DRYING (731): Dry a sample at 105° for 3 h; it loses NMT 0.4% of its weight.

ADDITIONAL REQUIREMENTS

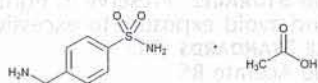
• PACKAGING AND STORAGE: Preserve in well-closed containers.

• USP REFERENCE STANDARDS (11)

USP Arginine Hydrochloride RS

USP L-Lysine Hydrochloride RS

Mafenide Acetate



$C_7H_{10}N_2O_2S \cdot C_2H_4O_2$ 246.28
Benzenesulfonamide, 4-(aminomethyl)-, monoacetate;
 α -Amino-*p*-toluenesulfonamide monoacetate [13009-99-9].

DEFINITION

Mafenide Acetate contains NLT 98.0% and NMT 102.0% of mafenide acetate ($C_7H_{10}N_2O_2S \cdot C_2H_4O_2$), calculated on the anhydrous basis.

IDENTIFICATION

- A. INFRARED ABSORPTION (197K)**
- B.** The R_f value of the principal spot of the *Identification solution* corresponds to that of *Standard solution A*, as obtained in the test for *Organic Impurities*.

ASSAY

PROCEDURE

Standard solution: 200 μ g/mL of USP Mafenide Acetate RS in 0.01 N hydrochloric acid

Sample stock solution: 2 mg/mL of Mafenide Acetate

Sample solution: 200 μ g/mL of Mafenide Acetate prepared as follows. Pipet 10 mL of the *Sample stock solution* into a 100-mL volumetric flask containing 1 mL of 1 N hydrochloric acid, and dilute with water to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: 267 nm

Cell: 1 cm

Blank: 0.01 N hydrochloric acid

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of mafenide acetate ($C_7H_{10}N_2O_2S \cdot C_2H_4O_2$) in the portion of Mafenide Acetate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Mafenide Acetate RS in the *Standard solution* (μ g/mL)

C_U = concentration of Mafenide Acetate in the *Sample solution* (μ g/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- RESIDUE ON IGNITION (281):** NMT 0.2%

- SELENIUM (291)**

Sample: 200 mg

Acceptance criteria: NMT 30 ppm

Delete the following:

- HEAVY METALS, Method II (231):** NMT 20 ppm • (Official 1-

Jan-2018)

- ORGANIC IMPURITIES**

Standard solution A: 500 μ g/mL of USP Mafenide Acetate RS in methanol

Standard solution D: 500 μ g/mL of USP Mafenide Related Compound A RS in methanol

[NOTE—USP Mafenide Related Compound A RS is 4-formylbenzenesulfonamide.]

Standard solutions: Quantitatively dilute portions of *Standard solution A* with methanol to obtain *Standard solution B* and *Standard solution C*. Similarly, quantitatively dilute portions of *Standard solution D* with metha-

nol to obtain *Standard solution E* and *Standard solution F*. The compositions are shown in Table 1.

Table 1

Standard Solution	Dilution	Concentration (μ g/mL)	Percentage (% for comparison with test specimen)
A	(undiluted)	500	1.0
B	5 in 10	250	0.5
C	1 in 5	100	0.2
D	(undiluted)	500	1.0
E	5 in 10	250	0.5
F	1 in 5	100	0.2

Sample solution: 50 mg/mL of Mafenide Acetate in methanol

Identification solution: 500 μ g/mL from the *Sample solution* in methanol

Ninhydrin solution: 300 mg of ninhydrin in 100 mL of butyl alcohol. Add 3 mL of glacial acetic acid.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Developing solvent system: Ethyl acetate, methanol, and isopropylamine (77:20:3)

Spray reagent: *Ninhydrin solution*

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Identification solution*

Apply the *Samples* separately to the chromatographic plate. Position the plate in a chromatographic chamber, and develop the chromatograms in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in warm, circulating air. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution* at the R_f value corresponding to those of the principal spots in the chromatograms of *Standard solutions D*, *E*, and *F*. Spray the plate with *Spray reagent*, heat the plate at 105° for 5 min, and examine the plate. Compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution* to those of the principal spots in the chromatograms of *Standard solutions A*, *B*, and *C*.

Acceptance criteria: No secondary spot, observed by both visualizations, from the chromatogram of the *Sample solution* is larger or more intense than the principal spots obtained from *Standard solution B* (0.5%) and *Standard solution E* (0.5%). The sum of the intensities of all secondary spots obtained from the *Sample solution* corresponds to NMT 1.0%.

SPECIFIC TESTS

- MELTING RANGE OR TEMPERATURE (741):** Between 162° and 171°, but the range between the beginning and end of melting does not exceed 4°.

- PH (791)**

Sample solution: 100 mg/mL

Acceptance criteria: 6.4–6.8

- WATER DETERMINATION, Method I (921):** NMT 1.0%

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

- **USP REFERENCE STANDARDS (11)**
USP Mafenide Acetate RS
USP Mafenide Related Compound A RS
4-Formylbenzenesulfonamide.
 $C_7H_7NO_3S$ 185.20

Mafenide Acetate Cream

DEFINITION

Mafenide Acetate Cream is Mafenide Acetate in a water-miscible, oil-in-water cream base, containing suitable preservatives. It contains NLT 90.0% and NMT 110.0% of mafenide acetate ($C_7H_{10}N_2O_2S \cdot C_2H_4O_2$), in terms of the labeled amount of mafenide ($C_7H_{10}N_2O_2S$).

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION (197U)**
Sample solution: Proceed as directed in the Assay.
Acceptance criteria: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL, Acetate (191)**
Sample solution: Place about 1 g in a 60-mL separatory funnel, and add 20 mL of chloroform to dissolve it. Add 20 mL of water, shake for 2 min, allow the layers to separate completely, and discard the lower chloroform layer. Repeat this washing with another 20-mL portion of chloroform, and discard the chloroform washing. Centrifuge the aqueous layer. Use a 1-mL aliquot of the supernatant with 2 mL of water for the lanthanum nitrate test and a 3-mL aliquot of the supernatant for the ferric chloride test.
Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Standard solution: 200 μ g/mL of USP Mafenide Acetate RS in 0.01 N hydrochloric acid

Sample solution: Nominally 200 μ g/mL of mafenide acetate prepared as follows. Transfer a portion of Cream containing 100 mg of mafenide acetate to a 60-mL separator, and add 20 mL of chloroform to dissolve it. Add 20 mL of water, shake for 2 min, allow the layers to separate completely, and discard the lower chloroform layer. Repeat this washing with two separate 20-mL portions of chloroform, and discard the chloroform washings. Pass the aqueous phase through a dry filter into a 100-mL volumetric flask. Rinse the separator and the filter with water, passing all rinses through the filter, and add water to volume. Centrifuge 30 mL, then pipet 20 mL of the clear supernatant into a 100-mL volumetric flask. Add 1 mL of 1 N hydrochloric acid, and add water to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: 267 nm

Cell: 1 cm

Blank: 0.01 N hydrochloric acid

Analysis

Samples: Standard solution, Sample solution, and Blank
Calculate the percentage of the labeled amount of mafenide acetate ($C_7H_{10}N_2O_2S \cdot C_2H_4O_2$) in the portion of Cream taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the Sample solution

A_S = absorbance of the Standard solution

C_S = concentration of USP Mafenide Acetate RS in the Standard solution (μ g/mL)

C_U = nominal concentration of mafenide acetate in the Sample solution (μ g/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS (11)**
USP Mafenide Acetate RS

Mafenide Acetate for Topical Solution

DEFINITION

Mafenide Acetate for Topical Solution contains NLT 98.0% and NMT 102.0% of mafenide acetate ($C_7H_{10}N_2O_2S \cdot C_2H_4O_2$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: Dissolve 6.8 g of monobasic potassium phosphate and 1.0 g of sodium 1-hexanesulfonate in 800 mL of water. Adjust with phosphoric acid to a pH of 2.5, and dilute to 1000 mL.

Mobile phase: Acetonitrile and Solution A (1:9)

Standard solution: 1 mg/mL of USP Mafenide Acetate RS in Mobile phase. Sonicate to dissolve if necessary.

Sample solution: Nominally 1 mg/mL of mafenide acetate prepared as follows. Constitute the Topical Solution as directed in the labeling. Transfer a volume of the constituted Topical Solution, equivalent to 25 mg of mafenide acetate, to a 25-mL volumetric flask. Using sonication, dissolve in 12 mL of Mobile phase. Dilute with Mobile phase to volume.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 267 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: Standard solution

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of mafenide acetate ($C_7H_{10}N_2O_2S \cdot C_2H_4O_2$) in the portion of the constituted Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Mafenide Acetate RS in the Standard solution (mg/mL)

C_U = nominal concentration of mafenide acetate in the Sample solution (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

OTHER COMPONENTS

• CONTENT OF ACETIC ACID

Internal standard solution: 0.5% (v/v) of propionic acid in water

Standard stock solution: Transfer 50 mL of water to a 100-mL volumetric flask, insert a stopper, and weigh. Add 0.5 mL of glacial acetic acid to the flask, insert the

stopper, weigh, and calculate, by difference, the amount of acetic acid added. Dilute with water to volume.

Standard solution: 530 µg/mL of acetic acid prepared as follows. Add 10.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution* to a 100-mL volumetric flask containing 200 mg of oxalic acid. Dilute with water to volume.

Sample solution: Nominally 2 mg/mL of mafenide acetate prepared as follows. Constitute the Topical Solution as directed in the labeling. Transfer a volume of the constituted Topical Solution, equivalent to 200 mg of mafenide acetate, to a 100-mL volumetric flask containing 200 mg of oxalic acid. Pipet 10.0 mL of *Internal standard solution* into the flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 60-m fused-silica capillary column coated with a 0.5-µm layer of acid-deactivated phase G35

Temperatures

Injection: 250°

Detector: 250°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
150	0	150	11
150	25	240	10
240	25	150	1

Carrier gas: Helium

Flow rate: 40 cm/s

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between acetic acid and propionic acid

Relative standard deviation: NMT 6.0% for peak response ratios

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acetic acid in the portion of the constituted Topical Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of acetic acid to propionic acid from the *Sample solution*

R_S = peak response ratio of acetic acid to propionic acid from the *Standard solution*

C_S = concentration of acetic acid in the *Standard solution* (mg/mL)

C_U = nominal concentration of mafenide acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 22.0%–26.8%

IMPURITIES

• ORGANIC IMPURITIES

Solution A: Dissolve 6.8 g of monobasic potassium phosphate and 1.0 g of sodium 1-hexanesulfonate in 800 mL of water. Adjust with phosphoric acid to a pH of 2.5, and dilute to 1000 mL.

Mobile phase: Acetonitrile and *Solution A* (1:9)

Standard stock solution: 25 µg/mL of USP Mafenide Related Compound A RS in *Mobile phase*

[NOTE—USP Mafenide Related Compound A RS is 4-formylbenzenesulfonamide.]

System suitability solution: 1.0 mg/mL of USP Mafenide Acetate RS and 10 µg/mL of USP Mafenide Related Compound A RS from *Standard stock solution* in *Mobile phase*. Initially dissolve the USP Mafenide Acetate RS using 20% of the final volume by sonication in 2 mL of *Mobile phase*, add the appropriate volume of USP Mafenide Related Compound A RS, and dilute to final volume.

Standard solution A: 5 µg/mL of USP Mafenide Related Compound A RS from *Standard stock solution* in *Mobile phase*

Standard solution B: 1 µg/mL of USP Mafenide Related Compound A RS from *Standard solution A* in *Mobile phase*

Sample solution: Proceed as directed in the Assay.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 267 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 3 times the retention time of mafenide

System suitability

Samples: *System suitability solution*, *Standard solution A*, and *Standard solution B*

Suitability requirements

Resolution: NLT 3.0 between mafenide acetate and mafenide related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution A*

Analysis

Samples: *Mobile phase*, *Standard solution A*, and *Sample solution*

Chromatograph the *Standard solution* and adjust the integration parameters so that the response is 5%–15% of full-scale deflection. Disregard the peaks corresponding to those obtained from the *Mobile phase*. Calculate the percentage of each impurity in the portion of the constituted Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response of mafenide related compound A from *Standard solution A*

C_S = concentration of USP Mafenide Related Compound A RS in *Standard solution A* (µg/mL)

C_U = nominal concentration of mafenide acetate in the *Sample solution* (µg/mL)

Acceptance criteria

Individual impurity: NMT 0.5%

Total impurities: NMT 1.0%

SPECIFIC TESTS

• pH (791)

Sample solution: Nominally 100 mg/mL

Acceptance criteria: 6.4–6.8

• WATER DETERMINATION, Method I (921): NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, at controlled room temperature. For prepared solutions, use within 48 h of preparation.

• USP REFERENCE STANDARDS (11)

USP Mafenide Acetate RS

USP Mafenide Related Compound A RS

4-Formylbenzenesulfonamide.

C₇H₇NO₃S 185.20

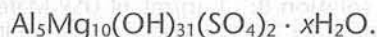
Magaldrate

Aluminum magnesium hydroxide sulfate ($\text{Al}_5\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2 \cdot x\text{H}_2\text{O}$).

Aluminum magnesium hydroxide sulfate, hydrate
[74978-16-8].

Anhydrous 1097.38

» Magaldrate is a chemical combination of aluminum and magnesium hydroxides and sulfate, corresponding approximately to the formula:



It contains the equivalent of not less than 90.0 percent and not more than 105.0 percent of $\text{Al}_5\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—
USP Magaldrate RS

Identification—

A: Dissolve about 600 mg in 20 mL of 3 N hydrochloric acid, add 3 drops of methyl red TS and about 30 mL of water, and heat to boiling. Add 6 N ammonium hydroxide until the color just changes to yellow, continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* (191).

B: Wash the precipitate obtained in *Identification* test A with 50 mL of hot ammonium chloride solution (1 in 50), then dissolve the precipitate in 15 mL of 3 N hydrochloric acid: the solution responds to the tests for *Aluminum* (191).

C: Its X-ray diffraction pattern (see *X-ray Diffraction* (941)) in the d-spacings region below 0.257 nm (2.57 angstrom units) conforms to that of USP Magaldrate RS.

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the test for absence of *Escherichia coli*.

Loss on drying (731)—Dry it at 200° for 4 hours: it loses between 10.0% and 20.0% of its weight.

Soluble chloride—Boil 1 g of it, accurately weighed, with 50.0 mL of water for 5 minutes, cool, add water to restore the original volume, mix, and filter. To 25.0 mL of the filtrate add 0.1 mL of potassium chromate TS, and titrate with 0.10 N silver nitrate until a persistent pink color is obtained: not more than 5.0 mL of 0.10 N silver nitrate is required (3.5%).

Soluble sulfate (221)—A 2.5-mL portion of the filtrate obtained in the test for *Soluble chloride* shows no more sulfate than corresponds to 1.0 mL of 0.020 N sulfuric acid (1.9%).

Sodium—Transfer 2 g of it, accurately weighed, to a 100-mL volumetric flask, place in an ice bath, add 5 mL of nitric acid, and swirl to dissolve. Allow to warm to room temperature, dilute with water to volume, and mix. Filter, if necessary, to obtain a clear solution. Dilute 10.0 mL of the filtrate with water to 100.0 mL: the emission intensity of this solution, determined with a suitable flame photometer at 589 nm and corrected for background transmission at 580 nm, is not greater than that produced by a standard containing 2.2 µg of Na per mL, similarly measured (0.11%).

Arsenic, Method I (211): 8 ppm.

Delete the following:

• **Heavy metals** (231)—Dissolve 330 mg in 10 mL of 3 N hydrochloric acid, filter if necessary to obtain a clear solu-

tion, and dilute with water to 25 mL: the limit is 0.006%.

• (Official 1-Jan-2018)

Magnesium hydroxide content—Dissolve about 100 mg, accurately weighed, in 3 mL of dilute hydrochloric acid (1 in 10), and dilute with water to about 200 mL. Add, with stirring, 1 g of ammonium chloride, 20 mL of triethanolamine, 10 mL of ammonia–ammonium chloride buffer TS, and 0.1 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue color. Perform a blank determination, and make any necessary correction. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of $\text{Mg}(\text{OH})_2$: between 49.2% and 66.6% of $\text{Mg}(\text{OH})_2$ is found, calculated on the dried basis.

Aluminum hydroxide content—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Procedure—Dissolve about 100 mg of Magaldrate, accurately weighed, in 3 mL of dilute hydrochloric acid (1 in 10), and dilute with water to about 30 mL. Add, with stirring, 25.0 mL of *Edetate disodium titrant*, mix, and allow to stand for 5 minutes. Then add 20 mL of acetic acid–ammonium acetate buffer TS, 60 mL of alcohol, and 2 mL of dithizone TS, and titrate with 0.05 M zinc sulfate to a bright rose-pink color. Perform a blank determination, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of $\text{Al}(\text{OH})_3$: between 32.1% and 45.9% of $\text{Al}(\text{OH})_3$ is found, calculated on the dried basis.

Sulfate content—

Chromatographic column—Transfer 15 mL of strongly acidic 50- to 100-mesh styrene-divinylbenzene cation-exchange resin to a 1-cm inside diameter glass column. Wash the resin with 30 mL of water.

Indicator solution—Prepare a solution in water containing 2 mg of sodium alizarinsulfonate per mL.

Magnesium acetate solution—Dissolve 26.8 g of magnesium acetate in 500 mL of water.

0.05 M Barium chloride—Dissolve 12.2 g of barium chloride in about 900 mL of water, adjust with 1 N hydrochloric acid to a pH of 3.0, dilute with water to 1000 mL, and mix. Standardize this solution as follows: Transfer 10.0 mL of 0.1 N sulfuric acid VS to a 125-mL conical flask. Adjust by adding *Magnesium acetate solution* to a pH of 3.0. Add 25 mL of methanol and 3 or 4 drops of *Indicator solution*. Add from a buret an accurately measured volume of 8 to 9 mL of 0.05 M barium chloride. Add an additional 4 drops of *Indicator solution*, and titrate slowly until the yellow color disappears and a pink tinge is visible. Calculate the molarity of the barium chloride titrant taken by the formula:

$$5(N/V)$$

in which *N* is the normality of the sulfuric acid; and *V* is the volume, in mL, of titrant consumed.

Test preparation—Transfer about 875 mg of Magaldrate, accurately weighed, to a 25-mL volumetric flask. Dissolve in 10 mL of water and 5 mL of glacial acetic acid, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to the chromatographic column and wash the column with 15 mL of water, collecting the eluate in a 125-mL conical flask (*Test preparation*).

Procedure—Add to the *Test preparation* 5 mL of *Magnesium acetate solution*, 32 mL of methanol, and 3 or 4 drops of *Indicator solution*. Add from a buret an accurately measured volume of 5.0 to 5.5 mL of 0.05 M barium chloride. Add an additional 3 drops of *Indicator solution*, and titrate slowly until the yellow color disappears and a pink tinge is visible. Each mL of 0.05 M barium chloride is equivalent to 4.803 mg of sulfate (SO_4): between 16.0% and 21.0% of SO_4 is found, calculated on the dried basis.

Assay—Transfer about 3 g of Magaldrate, accurately weighed, to a 250-mL beaker, add 100.0 mL of 1 N hydrochloric acid VS, and stir until the solution becomes clear.

Titrate the excess acid with 1 N sodium hydroxide VS to a pH of 3.0, determined potentiometrically. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 1 N hydrochloric acid is equivalent to 35.40 mg of $\text{Al}_3\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2$.

Magaldrate Oral Suspension

» Magaldrate Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of magaldrate $[\text{Al}_3\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2]$.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Magaldrate RS

Identification—

A: Dissolve an amount of Oral Suspension, equivalent to about 800 mg of magaldrate, in 20 mL of 3 N hydrochloric acid, dilute with water to about 50 mL, add 3 drops of methyl red TS, and proceed as directed in *Identification test A* under *Magaldrate*, beginning with "and heat to boiling."

B: It responds to *Identification test B* under *Magaldrate*.

C: Transfer an amount of Oral Suspension, equivalent to about 1 g of magaldrate, to a 100-mL centrifuge tube. Add about 60 mL of water, cap, and shake for 3 minutes. Centrifuge the suspension, and discard the supernatant. Repeat the washing of the residue with three 60-mL portions of water. Transfer the residue to a 250-mL beaker, and heat on a steam bath to dryness: the X-ray diffraction pattern (see *X-ray Diffraction* (941)), in the d-spacings region below 2.57 angstrom units, of the residue so obtained conforms to that of USP Magaldrate RS.

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for absence of *Escherichia coli*.

Acid-neutralizing capacity (301)—The acid consumed by the minimum single dose recommended in the labeling is not less than 5 mEq, and not less than the number of mEq calculated by the formula:

$$0.8(0.0282M)$$

in which 0.0282 is the theoretical acid-neutralizing capacity, in mEq per mg, of magaldrate, and M is the quantity, in mg, of the labeled amount of magaldrate.

Magnesium hydroxide content—

Test preparation—Transfer an accurately measured quantity of Oral Suspension, equivalent to about 1 g of magaldrate, to a 100-mL volumetric flask, add 30 mL of dilute hydrochloric acid (1 in 10), shake to dissolve, dilute with water to volume, and mix.

Procedure—Transfer 10.0 mL of *Test preparation* to a 400-mL beaker, and proceed as directed in the test for *Magnesium hydroxide content* under *Magaldrate*, beginning with "and dilute with water to about 200 mL." Not less than 492 mg and not more than 666 mg of magnesium hydroxide $[\text{Mg}(\text{OH})_2]$ per g of the labeled amount of magaldrate is found.

Aluminum hydroxide content—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Test preparation—Prepare as directed in the test for *Magnesium hydroxide content*.

Procedure—Transfer 10.0 mL of *Test preparation* and 20 mL of water to a 250-mL beaker, and proceed as di-

rected for *Procedure* in the test for *Aluminum hydroxide content* under *Magaldrate*, beginning with "Add, with stirring, 25.0 mL of *Edetate disodium titrant*." Not less than 321 mg and not more than 459 mg of aluminum hydroxide $[\text{Al}(\text{OH})_3]$ per g of the labeled amount of magaldrate is found.

Change to read:

Other requirements—Evaporate a volume of Oral Suspension, equivalent to about 5 g of magaldrate, on a steam bath to dryness: the residue so obtained meets the requirements of the tests for *Arsenic* (Official 1-Jan-2018) under *Magaldrate*.

Assay—Transfer an accurately measured quantity of Oral Suspension, equivalent to about 3 g of magaldrate, to a beaker. Add 100.0 mL of 1 N hydrochloric acid VS, and mix, using a magnetic stirrer to achieve dissolution. Titrate the excess acid with 1 N sodium hydroxide VS to a pH of 3.0, determined potentiometrically. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 1 N hydrochloric acid is equivalent to 35.40 mg of $\text{Al}_3\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2$.

Magaldrate Tablets

» Magaldrate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of magaldrate $[\text{Al}_3\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2]$.

Packaging and storage—Preserve in well-closed containers.

Labeling—Label the Tablets to indicate whether they are to be swallowed or to be chewed.

USP Reference standards (11)—

USP Magaldrate RS

Identification—Transfer a quantity of powdered Tablets, equivalent to about 2 g of magaldrate, to a 100-mL centrifuge tube. Add about 60 mL of water, cap, and shake for 3 minutes. Centrifuge the suspension, and discard the supernatant. Repeat the washing with three more 60-mL portions of water. Transfer the residue to a 250-mL beaker, and heat on a steam bath to dryness: the residue so obtained meets the requirements of the *Identification* tests under *Magaldrate*.

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—Tablets meet the requirements of the test for absence of *Escherichia coli*.

Disintegration (701): 2 minutes, for Tablets labeled to be swallowed.

Uniformity of dosage units (905): meet the requirements for *Weight Variation*.

Acid-neutralizing capacity—Proceed as directed under *Acid-Neutralizing Capacity* (301). The acid consumed by the minimum single dose recommended in the labeling is not less than 5 mEq, and not less than the number of mEq calculated by the formula:

$$0.8(0.0282M)$$

in which 0.0282 is the theoretical acid-neutralizing capacity, in mEq per mg, of magaldrate; and M is the quantity, in mg, of the labeled amount of magaldrate.

Magnesium hydroxide content—

Test preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 g of magaldrate, to a

100-mL volumetric flask, add 30 mL of dilute hydrochloric acid (1 in 10), shake for 15 minutes, dilute with water to volume, and mix.

Procedure—Transfer 10.0 mL of *Test preparation* to a 400-mL beaker, and proceed as directed in the test for *Magnesium hydroxide content* under *Magaldrate*, beginning with “and dilute with water to about 200 mL.” Not less than 492 mg and not more than 666 mg of magnesium hydroxide $[\text{Mg}(\text{OH})_2]$ per g of the labeled amount of magaldrate is found.

Aluminum hydroxide content—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Test preparation—Prepare as directed in the test for *Magnesium hydroxide content*.

Procedure—Transfer 10.0 mL of *Test preparation* and 20 mL of water to a 250-mL beaker, and proceed as directed for *Procedure* in the test for *Aluminum hydroxide content* under *Magaldrate*, beginning with “Add, with stirring, 25.0 mL of *Edetate disodium titrant*.” Not less than 321 mg and not more than 459 mg of aluminum hydroxide $[\text{Al}(\text{OH})_3]$ per g of the labeled amount of magaldrate is found.

Assay—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 6 g of magaldrate, to a 200-mL volumetric flask. Add 100.0 mL of 2 N hydrochloric acid VS, and swirl by mechanical means for 30 minutes. Dilute with water to volume, mix, and filter. Transfer 100.0 mL of the filtrate to a beaker. Titrate the excess acid with 1 N sodium hydroxide VS to a pH of 3.0, determined potentiometrically. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 2 N hydrochloric acid is equivalent to 70.80 mg of $\text{Al}_5\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2$.

Magaldrate and Simethicone Oral Suspension

» Magaldrate and Simethicone Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of magaldrate $[\text{Al}_5\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2]$, and an amount of polydimethylsiloxane $[-(\text{CH}_3)_2\text{SiO}-]_n$ that is not less than 85.0 percent and not more than 115.0 percent of the labeled amount of simethicone.

Packaging and storage—Preserve in tight containers, and keep from freezing.

USP Reference standards (11)—

USP Magaldrate RS
USP Polydimethylsiloxane RS

Identification—

A: Dissolve an amount of Oral Suspension, equivalent to about 800 mg of magaldrate, in 20 mL of 3 N hydrochloric acid, dilute with water to about 50 mL, add 3 drops of methyl red TS, and proceed as directed in *Identification test A* under *Magaldrate*, beginning with “and heat to boiling.”

B: Wash the precipitate obtained in *Identification test A* with hot ammonium chloride solution (1 in 50), and dissolve the precipitate in hydrochloric acid. Divide this solution into two portions: the dropwise addition of 6 N ammonium hydroxide to one portion yields a gelatinous white precipitate, which does not dissolve in an excess of 6 N ammonium hydroxide. The dropwise addition of 1 N sodium hydroxide to the other portion yields a gelatinous white pre-

cipitate, which dissolves in an excess of 1 N sodium hydroxide, leaving some turbidity.

C: Transfer an amount of Oral Suspension, equivalent to about 1 g of magaldrate, to a 100-mL centrifuge tube. Add about 60 mL of water, insert the cap, and shake for 3 minutes. Centrifuge the suspension, and discard the supernatant. Repeat the washing of the residue with three 60-mL portions of water. Transfer the residue to a 250-mL beaker, and heat on a steam bath to dryness: the X-ray diffraction pattern (see *X-ray Diffraction* (941)), in the d-spacings region below 2.57 angstrom units, of the residue so obtained conforms to that of USP Magaldrate RS.

D: The IR absorption spectrum, in the 7- to 15- μm region, determined in a 0.1-mm cell, of the *Assay preparation* prepared as directed in the *Assay* for polydimethylsiloxane exhibits maxima only at the same wavelengths as that of the *Standard preparation* prepared as directed in the *Assay* for polydimethylsiloxane.

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for absence of *Escherichia coli*.

Acid-neutralizing capacity (301)—The acid capacity by the minimum single dose recommended in the labeling is not less than 5 mEq, and not less than the number of mEq calculated by the formula:

$$0.8(0.0282M)$$

in which 0.0282 is the theoretical acid-neutralizing capacity, in mEq per mg, of magaldrate; and *M* is the quantity, in mg, of the labeled amount of magaldrate.

Magnesium hydroxide content—

Test preparation—Transfer an accurately measured quantity of Oral Suspension, equivalent to about 1 g of magaldrate, to a 100-mL volumetric flask, add 30 mL of dilute hydrochloric acid (1 in 10), shake to dissolve, dilute with water to volume, and mix.

Procedure—Transfer 10.0 mL of *Test preparation* to a 400-mL beaker, and proceed as directed in the test for *Magnesium hydroxide content* under *Magaldrate*, beginning with “and dilute with water to about 200 mL.” Not less than 492 mg and not more than 666 mg of magnesium hydroxide $[\text{Mg}(\text{OH})_2]$ per g of the labeled amount of magaldrate is found.

Aluminum hydroxide content—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Test preparation—Prepare as directed in the test for *Magnesium hydroxide content*.

Procedure—Transfer 10.0 mL of *Test preparation* and 20 mL of water to a 250-mL beaker, and proceed as directed for *Procedure* in the test for *Aluminum hydroxide content* under *Magaldrate*, beginning with “Add, with stirring, 25.0 mL of *Edetate disodium titrant*.” Not less than 321 mg and not more than 459 mg of aluminum hydroxide $[\text{Al}(\text{OH})_3]$ per g of the labeled amount of magaldrate is found.

Change to read:

Other requirements—Evaporate a volume of Oral Suspension, equivalent to about 5 g of magaldrate, on a steam bath to dryness: the residue so obtained meets the requirements of the tests for *Arsenic* (Official: 1-Jan-2018) under *Magaldrate*.

Assay for magaldrate—Transfer an accurately measured quantity of Oral Suspension, equivalent to about 3 g of magaldrate, to a beaker. Add 100.0 mL of 1 N hydrochloric acid VS, and mix, using a magnetic stirrer to achieve dissolution. Titrate the excess acid with 1 N sodium hydroxide VS

to a pH of 3.0, determined potentiometrically. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 1 N hydrochloric acid is equivalent to 35.40 mg of magaldrate $[\text{Al}_5\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2]$.

Assay for polydimethylsiloxane—Transfer an accurately measured quantity of Oral Suspension, equivalent to about 250 mg of simethicone, to a 200-mL centrifuge bottle. Add an equal volume of hydrochloric acid, swirl to dissolve the Oral Suspension, add 25.0 mL of hexanes, and immediately close the bottle securely with a cap having an inert liner. Shake the bottle for 30 minutes, and centrifuge the mixture until a clear supernatant layer is obtained (*Assay preparation*). Prepare a *Standard preparation* of USP Polydimethylsiloxane RS in hexanes having a known concentration of about 10 mg per mL. Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 0.1-mm cells at the wavelength of maximum absorbance at about 7.9 μm and at the wavelengths of minimum absorbance at about 7.5 μm and 8.3 μm , with a suitable IR spectrophotometer, using hexanes as the blank. Draw a linear baseline between the two minima, and determine the absorbances for the *Standard preparation* and the *Assay preparation* with respect to the baseline, making any necessary correction for the blank. Calculate the quantity, in mg, of $[(\text{CH}_3)_2\text{SiO}]_n$ in the portion of Oral Suspension taken by the formula:

$$25C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Polydimethylsiloxane RS in the *Standard preparation*; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Magaldrate and Simethicone Chewable Tablets

Former Title: *Magaldrate and Simethicone Tablets*

» Magaldrate and Simethicone Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of magaldrate $[\text{Al}_5\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2]$, and an amount of polydimethylsiloxane $[(\text{CH}_3)_2\text{SiO}]_n$ that is not less than 85.0 percent and not more than 115.0 percent of the labeled amount of simethicone.

Packaging and storage—Preserve in well-closed containers.

Labeling—Label the Chewable Tablets to indicate that they are to be chewed before being swallowed.

USP Reference standards (11)—

USP Magaldrate RS
USP Polydimethylsiloxane RS

Identification—

A: Transfer a quantity of powdered Chewable Tablets, equivalent to about 2 g of magaldrate, to a 100-mL centrifuge tube. Add about 60 mL of water, cap, and shake for 3 minutes. Centrifuge the suspension, and discard the supernatant. Repeat the washing with three more 60-mL portions of water. Transfer the residue to a 250-mL beaker, and heat on a steam bath to dryness: the residue so obtained meets the requirements of the *Identification* tests under *Magaldrate*.

B: The IR absorption spectrum, in the 7- to 11- μm region, determined in a 0.5-mm cell, of the *Assay preparation* prepared as directed in the *Assay for polydimethylsiloxane*, exhibits maxima only at the same wavelengths as that of the *Standard preparation* containing about 2 mg of USP

Polydimethylsiloxane RS per mL prepared as directed in the *Assay for polydimethylsiloxane*.

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—Chewable Tablets meet the requirements of the test for absence of *Escherichia coli*.

Uniformity of dosage units (905): meet the requirements for *Weight Variation* with respect to magaldrate.

Acid-neutralizing capacity—Proceed as directed under *Acid-neutralizing Capacity* (301). The acid consumed by the minimum single dose recommended in the labeling is not less than 5 mEq, and not less than the number of mEq calculated by the formula:

$$0.8(0.0282 M)$$

in which 0.0282 is the theoretical acid-neutralizing capacity, in mEq per mg, of magaldrate, and M is the quantity, in mg, of the labeled amount of magaldrate.

Magnesium hydroxide content—

Test preparation—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 g of magaldrate, to a 100-mL volumetric flask, add 30 mL of dilute hydrochloric acid (1 in 10), shake for 15 minutes, dilute with water to volume, and mix.

Procedure—Transfer 10.0 mL of the *Test preparation* to a 400-mL beaker, and proceed as directed in the test for *Magnesium hydroxide content* under *Magaldrate*, beginning with "and dilute with water to about 200 mL." Not less than 492 mg and not more than 666 mg of magnesium hydroxide $[\text{Mg}(\text{OH})_2]$ per g of the labeled amount of magaldrate is found.

Aluminum hydroxide content—

Edetate disodium titrant—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

Test preparation—Prepare as directed in the test for *Magnesium hydroxide content*.

Procedure—Transfer 10.0 mL of *Test preparation* and 20 mL of water to a 250-mL beaker, and proceed as directed for *Procedure* in the test for *Aluminum hydroxide content* under *Magaldrate*, beginning with "Add, with stirring, 25.0 mL of *Edetate disodium*." Not less than 321 mg and not more than 459 mg of aluminum hydroxide $[\text{Al}(\text{OH})_3]$ per g of the labeled amount of magaldrate is found.

Assay for magaldrate—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 6 g of magaldrate, to a 200-mL volumetric flask. Add 100.0 mL of 2 N hydrochloric acid VS, and swirl by mechanical means for 30 minutes. Dilute with water to volume, mix, and filter. Transfer 100.0 mL of the filtrate to a beaker. Titrate the excess acid with 1 N sodium hydroxide VS to a pH of 3.0, determined potentiometrically. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 2 N hydrochloric acid is equivalent to 70.80 mg of $\text{Al}_5\text{Mg}_{10}(\text{OH})_{31}(\text{SO}_4)_2$.

Assay for polydimethylsiloxane—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of simethicone, to a 60-mL separator. Add 10.0 mL of hexanes and 25 mL of 6 N hydrochloric acid, cap the separator, and shake by mechanical means for not less than 2 hours. Allow to stand for about 10 minutes, and drain off as much of the lower, aqueous layer as possible without removing any of the unseparated interphase. Add 25 mL of 4 N sodium hydroxide to the separator, cap it, and shake by mechanical means for 1 hour. Transfer the mixture from the separator to a 50-mL centrifuge tube, cap, and centrifuge to obtain clear layers. Transfer not less than 5 mL of the clear upper hexanes layer to a test tube containing about 0.5 g of anhydrous sodium sulfate. Cap the tube, shake vigorously, and allow to stand to obtain a clear supernatant (*Assay*

preparation). Prepare three *Standard preparations* in hexanes having known concentrations of about 1.6, 2.0, and 2.4 mg of USP Polydimethylsiloxane RS per mL, respectively. Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparations* in a 0.5-mm cell at the wavelength of maximum absorbance at about 1260 cm^{-1} with an IR spectrophotometer, using hexanes as the blank. [NOTE—Between each measurement, rinse the cell with heptane, empty, and dry it.] Plot the absorbances for the *Standard preparations* versus concentration, in mg per mL, of USP Polydimethylsiloxane RS, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C , in mg per mL, of polydimethylsiloxane in the *Assay preparation*. Calculate the quantity, in mg, of $[-(\text{CH}_3)_2\text{SiO}-]_n$ in the portion of Chewable Tablets taken by multiplying C by 10.

Milk of Magnesia

$\text{Mg}(\text{OH})_2$ 58.32

Magnesium hydroxide.

Magnesium hydroxide [1309-42-8].

» Milk of Magnesia is a suspension of Magnesium Hydroxide. Milk of Magnesia, Double-Strength Milk of Magnesia, and Triple-Strength Milk of Magnesia contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $\text{Mg}(\text{OH})_2$, the labeled amount being 80, 160, and 240 mg of $\text{Mg}(\text{OH})_2$ per mL, respectively. It may contain not more than 0.05 percent of a volatile oil or a blend of volatile oils, suitable for flavoring purposes.

Packaging and storage—Preserve in tight containers, preferably at a temperature not exceeding 35°. Avoid freezing.

Labeling—Double- or Triple-Strength Milk of Magnesia is so labeled, or may be labeled as 2× or 3× Concentrated Milk of Magnesia, respectively.

Identification—A solution of the equivalent of 1 g of regular-strength Milk of Magnesia in 2 mL of 3 N hydrochloric acid meets the requirements of the tests for *Magnesium* (191).

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for absence of *Escherichia coli*.

Acid-neutralizing capacity (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than the number of mEq calculated by the formula:

$$0.8(0.0343M)$$

in which 0.0343 is the theoretical acid-neutralizing capacity, in mEq, of $\text{Mg}(\text{OH})_2$; and M is the quantity, in mg, of $\text{Mg}(\text{OH})_2$ in the specimen tested, based on the labeled quantity.

Soluble alkalies—Centrifuge about 50 mL of Milk of Magnesia. Dilute 5.0 mL of the clear supernatant with 40 mL of water. Add 1 drop of methyl red TS, and titrate the solution with 0.10 N sulfuric acid to the production of a persistent pink color: not more than 1.0 mL of the acid is required. Where the specimen is Double- or Triple-Strength Milk of Magnesia, not more than 2.0 or 3.0 mL of the acid is required, respectively.

Carbonate and acid-insoluble matter—To the equivalent of 1 g of regular-strength Milk of Magnesia add 2 mL of

3 N hydrochloric acid: not more than a slight effervescence occurs, and the solution is not more than slightly turbid.

Assay—Transfer an accurately measured quantity of Milk of Magnesia, previously shaken in its original container, equivalent to about 800 mg of magnesium hydroxide, to a 250-mL volumetric flask. Dissolve in 30 mL of 3 N hydrochloric acid, dilute with water to volume, and mix. Filter, if necessary, and transfer 25.0 mL of the filtrate to a beaker containing 75 mL of water, and mix. Adjust the reaction of the solution with 1 N sodium hydroxide to a pH of 7 (using pH indicator paper; see *Indicator and Test Papers* under *Reagents*, in the section *Reagents, Indicators, and Solutions*), add 5 mL of ammonia-ammonium chloride buffer TS and 0.15 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of $\text{Mg}(\text{OH})_2$.

Magnesia Tablets

DEFINITION

Magnesia Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of magnesium hydroxide $[\text{Mg}(\text{OH})_2]$.

IDENTIFICATION

• A. IDENTIFICATION TESTS—GENERAL, *Magnesium* (191)

Sample solution: Crush several Tablets, and dissolve 1 g of the powder in 20 mL of 3 N hydrochloric acid.

Acceptance criteria: Meet the requirements

ASSAY

• PROCEDURE

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 250 mg of magnesium hydroxide, to a 100-mL volumetric flask. Dissolve in 10 mL of 3 N hydrochloric acid, and dilute with water to volume. Filter, if necessary, and transfer 25.0 mL of the filtrate to a beaker containing 75 mL of water.

Analysis: Adjust the reaction of the solution with 1 N sodium hydroxide to a pH of 7 (using pH indicator paper; see *Reagents, Indicators, and Solutions—Indicator and Test Papers*), and add 5 mL of ammonia-ammonium chloride buffer TS and 0.15 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of $\text{Mg}(\text{OH})_2$.

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• DISINTEGRATION (701)

Time: NMT 10 min, simulated gastric fluid TS being substituted for water in the test

• UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

SPECIFIC TESTS

• ACID-NEUTRALIZING CAPACITY (301)

Analysis: NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated by the formula:

$$\text{Result} = (F_M \times M) \times 0.8$$

F_M = theoretical acid-neutralizing capacity of $\text{Mg}(\text{OH})_2$, 0.0343 mEq

M = quantity of $\text{Mg}(\text{OH})_2$ in the sample tested, based on the labeled quantity (mg)

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in well-closed containers.

Magnesium Carbonate

Carbonic acid, magnesium salt, basic; or, Carbonic acid, magnesium salt (1:1), hydrate; Magnesium carbonate, basic; or, Magnesium carbonate (1:1) hydrate [23389-33-5].

Anhydrous 84.31
[546-93-0].

DEFINITION

Magnesium Carbonate is a basic hydrated magnesium carbonate or a normal hydrated magnesium carbonate. It contains the equivalent of NLT 40.0% and NMT 43.5% of magnesium oxide (MgO).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Magnesium (191):** When treated with 3 N hydrochloric acid, it dissolves with effervescence, and the resulting solution meets the requirements.

ASSAY

• PROCEDURE

Sample: 1 g

Analysis: Dissolve the *Sample* in 30.0 mL of 1 N sulfuric acid VS, add methyl orange TS, and titrate the excess acid with 1 N sodium hydroxide VS. Perform the blank determination. Calculate the volume, V_s , of 1 N sulfuric acid, in mL, consumed by the *Sample*:

$$\text{Result} = (V_B - V_A) \times N_{\text{NaOH}}$$

V_B = volume of 1 N sodium hydroxide consumed by the blank determination (mL)

V_A = volume of 1 N sodium hydroxide consumed by the *Sample* (mL)

N_{NaOH} = exact normality of the sodium hydroxide solution

Calculate the volume of 1 N sulfuric acid, V_{Ca} , in mL, consumed by calcium, which is present in the portion of Magnesium Carbonate taken for the Assay:

$$\text{Result} = (W \times L_{\text{Ca}}) / (F_{\text{Ca}} \times 100)$$

W = weight of Magnesium Carbonate taken (mg)

L_{Ca} = content of calcium as determined in the test for Limit of Calcium (%)

F_{Ca} = weight of Ca that is equivalent to each mL of 1 N sulfuric acid, 20.04 mg

Calculate the percentage of magnesium oxide (MgO) in the portion of Magnesium Carbonate taken:

$$\text{Result} = (V_s - V_{\text{Ca}}) \times F_{\text{MgO}} / W \times 100$$

V_s = volume of 1 N sulfuric acid consumed by the *Sample*, as calculated above (mL)

V_{Ca} = volume of 1 N sulfuric acid consumed by calcium, as calculated above (mL)

F_{MgO} = weight of MgO that is equivalent to each mL of 1 N sulfuric acid, 20.15 mg

W = weight of Magnesium Carbonate taken (mg)

Acceptance criteria: 40.0%–43.5% of MgO

IMPURITIES

• SOLUBLE SALTS

Sample: 2.0 g

Analysis: Mix the *Sample* with 100 mL of a mixture of equal volumes of *n*-propyl alcohol and water. Heat the mixture to the boiling point with constant stirring, cool to room temperature, dilute with water to 100 mL, and filter. Evaporate 50 mL of the filtrate on a steam bath to dryness, and dry at 105° for 1 h.

Acceptance criteria: The weight of the residue does not exceed 10 mg (NMT 1.0%).

• ACID-INSOLUBLE SUBSTANCES

Sample: 5.0 g

Analysis: Mix the *Sample* with 75 mL of water, add hydrochloric acid in small portions, with agitation, until no more of the magnesium carbonate dissolves, and boil for 5 min. If an insoluble residue remains, filter, wash well with water until the last washing is free from chloride, and ignite.

Acceptance criteria: The weight of the ignited residue does not exceed 2.5 mg (NMT 0.05%).

• ARSENIC, Method I (211)

Test preparation: 750 mg in 25 mL of 3 N hydrochloric acid

Acceptance criteria: NMT 4 ppm

• LIMIT OF CALCIUM

[NOTE—A commercially available atomic absorption standard solution for calcium may be used where preparation of a calcium standard stock solution is described below. Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the instrument.]

Dilute hydrochloric acid: Dilute 100 mL of hydrochloric acid with water to 1000 mL.

Lanthanum solution: To 58.65 g of lanthanum oxide add 400 mL of water, and add, gradually with stirring, 250 mL of hydrochloric acid. Stir until dissolved, and dilute with water to 1000 mL.

Standard solutions: Transfer 249.7 mg of calcium carbonate, previously dried at 300° for 3 h and cooled in a desiccator for 2 h, to a 100-mL volumetric flask. Dissolve in a minimum amount of hydrochloric acid, and dilute with water to volume. Transfer 1.0, 5.0, 10.0, and 15.0 mL of this stock solution to separate 1000-mL volumetric flasks, each containing 20 mL of *Lanthanum solution* and 40 mL of *Dilute hydrochloric acid*. Dilute with water to volume. These *Standard solutions* contain 1.0, 5.0, 10.0, and 15.0 µg/mL of calcium, respectively.

Blank solution: Transfer 4 mL of *Lanthanum solution* and 10 mL of *Dilute hydrochloric acid* to a 200-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 250 mg of Magnesium Carbonate to a beaker, add 30 mL of *Dilute hydrochloric acid*, and stir until dissolved, heating if necessary. Transfer the solution so obtained to a 200-mL volumetric flask containing 4 mL of *Lanthanum solution*, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Lamp: Calcium hollow-cathode

Flame: Nitrous oxide–acetylene

Analytical wavelength: Calcium emission line at 422.7 nm

Analysis

Samples: *Standard solutions*, *Blank solution*, and *Sample solution*

Using the *Blank solution* as blank, determine the concentration, C , in µg/mL, of calcium in the *Sample solution* using the calibration graph.

Calculate the percentage of calcium in the portion of Magnesium Carbonate taken:

$$\text{Result} = (V/W \times C \times F) \times 100$$

V = volume of the *Sample solution* (mL)

W = weight of Magnesium Carbonate taken (mg)

C = as defined above

F = conversion factor from µg/mL to mg/mL, 0.001

Acceptance criteria: NMT 0.45%

Delete the following:

• HEAVY METALS, Method I (231)

Test preparation: Dissolve 0.67 g in 10 mL of 3 N hydrochloric acid in a suitable crucible, and evaporate the solution on a steam bath to dryness. Ignite at $550 \pm 25^\circ$ until all carbonaceous material is consumed. Dissolve the residue in 15 mL of water and 5 mL of hydrochloric acid, and evaporate to dryness. Toward the end of the evaporation, stir frequently to disintegrate the residue so that finally a dry powder is obtained. Dissolve the residue in 20 mL of water, and evaporate in the same manner as before to dryness. Redissolve the residue in 20 mL of water, filter, if necessary, and add to the filtrate 2 mL of 1 N acetic acid and water to make 25 mL.

Acceptance criteria: NMT 30 ppm (Official 1-Jan-2018)

• IRON (241)

Test preparation: Boil 50 mg with 5 mL of 2 N nitric acid for 1 min. Cool, dilute with water to 45 mL, add 2 mL of hydrochloric acid, and mix.

Acceptance criteria: NMT 200 ppm

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the test for absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Magnesium Carbonate and Citric Acid for Oral Solution

DEFINITION

Magnesium Carbonate and Citric Acid for Oral Solution contains a dry mixture of Magnesium Carbonate and Citric Acid that when constituted as directed in the labeling yields a solution that contains NLT 90.0% and NMT 110.0% of the labeled amount of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Magnesium (191)**
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: Meets the requirements
- **B.** The retention time of the citrate peak of the *Sample solution* corresponds to that of *Standard solution 1*, as obtained in the test for *Content of Anhydrous Citric Acid*.

ASSAY

• PROCEDURE

Sample solution: Transfer a volume of constituted oral solution, equivalent to 18.7 g of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$), to a 1000-mL volumetric flask. Add 200 mL of 1 N hydrochloric acid, swirl, and allow to stand for 10 min. Dilute with water to volume. Stir by mechanical means for 30 min.

Analysis: Transfer 10.0 mL of the *Sample solution* to a 250-mL beaker. Add 10 mL of ammonia-ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS until the last hint of violet disappears (blue endpoint). Each mL of 0.05 M edetate disodium is equivalent to 7.520 mg of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• CONTENT OF ANHYDROUS CITRIC ACID

Mobile phase, Standard solution 1, Chromatographic system, and System suitability: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate (345)*.

Sample solution: Transfer an appropriate volume of the constituted oral solution into a suitable volumetric flask, and proceed as directed for the *Sample solution* in *Assay for Citric Acid/Citrate and Phosphate (345)*, *Assay Preparation for Citric Acid/Citrate Assay*.

Analysis

Samples: *Standard solution 1* and *Sample solution*
Proceed as directed for *Assay for Citric Acid/Citrate and Phosphate (345)*, *Procedure*.

Calculate the percentage of anhydrous citric acid ($C_6H_8O_7$) in relation to the labeled amount of magnesium citrate in the volume of constituted oral solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of citrate from the *Sample solution*

r_S = peak area of citrate from *Standard solution 1*

C_S = concentration of citrate in *Standard solution 1* (mg/mL)

C_U = nominal concentration of magnesium citrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of anhydrous citric acid, 192.12

M_{r2} = molecular weight of citrate ($C_6H_5O_7$), 189.10

Acceptance criteria: 76.6%–107.8% of the labeled amount of magnesium citrate

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

IMPURITIES

• CHLORIDE AND SULFATE, Chloride (221)

Sample solution: Constitute as directed in the labeling.
Acceptance criteria: A 2.0-mL portion of *Sample solution* shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid (NMT 0.01%).

• CHLORIDE AND SULFATE, Sulfate (221)

Sample solution: Constitute as directed in the labeling.
Acceptance criteria: A 2.0-mL portion of *Sample solution* shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid (NMT 0.015%).

• TARTARIC ACID

Sample solution: Constitute as directed in the labeling.
Analysis: To 10 mL of *Sample solution* in a test tube, add 1 mL of glacial acetic acid and 3 mL of a solution of potassium acetate (1 in 2). Shake the mixture vigorously, then gently rub the inner wall of the test tube with a glass rod for a few min, and allow to stand for 1 h.

Acceptance criteria: No white, crystalline precipitate soluble in 6 N ammonium hydroxide is formed.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the test for absence of *Escherichia coli* and *Salmonella* species.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The label contains directions for constitution of the powder and states the equivalent amount of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$) in a given volume of the oral solution obtained after constitution.

- **USP REFERENCE STANDARDS (11)**
USP Citric Acid RS

Magnesium Carbonate, Citric Acid, and Potassium Citrate for Oral Solution

DEFINITION

Magnesium Carbonate, Citric Acid, and Potassium Citrate for Oral Solution contains a dry mixture of Magnesium Carbonate, Citric Acid, and Potassium Citrate that when constituted as directed in the labeling yields a solution that contains NLT 90.0% and NMT 110.0% of the labeled amount of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Magnesium (191)**
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: Meets the requirements
- **B.** The retention time of the citrate peak of the *Sample solution* corresponds to that of *Standard solution 1*, as obtained in the test for *Content of Anhydrous Citric Acid*.

ASSAY

• PROCEDURE

Sample solution: Transfer a volume of constituted oral solution, equivalent to 1.9 g of magnesium citrate to a 100-mL volumetric flask and dilute with water to volume.

Analysis: Transfer 10.0 mL of the *Sample solution* to a beaker. While stirring, add 10 mL of ammonia-ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS until the last hint of violet disappears (blue endpoint). Each mL of 0.05 M edetate disodium is equivalent to 7.520 mg of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• CONTENT OF ANHYDROUS CITRIC ACID

Mobile phase, Chromatographic system, and System suitability: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate (345)*.

Standard solution: 0.02 mg/mL of anhydrous citric acid, from USP Citric Acid RS in a freshly prepared 1 mM sodium hydroxide

Sample solution: Transfer a volume of constituted oral solution equivalent to 500 mg of magnesium citrate, to a suitable volumetric flask, and dilute quantitatively, and stepwise if necessary, with a freshly prepared 1 mM sodium hydroxide to obtain a solution having a concentration of 0.02 mg/mL of magnesium citrate, based on the label claim. Pass through a filter of 0.5- μ m or finer pore size, and use the filtrate.

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of anhydrous citric acid ($C_6H_8O_7$) in relation to the labeled amount of magnesium citrate in the volume of constituted oral solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak area of citrate from the *Sample solution*
 r_S = peak area of citrate from the *Standard solution*
 C_S = concentration of citrate in the *Standard solution* (mg/mL)
 C_U = nominal concentration of magnesium citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 126.1%–154.4% of the labeled amount of magnesium citrate

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

IMPURITIES

- **CHLORIDE AND SULFATE, Chloride (221)**
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: A 2.0-mL portion of *Sample solution* shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid (NMT 0.01%).
- **CHLORIDE AND SULFATE, Sulfate (221)**
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: A 2.0-mL portion of *Sample solution* shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid (NMT 0.015%).
- **TARTARIC ACID**
Sample solution: Constitute as directed in the labeling.
Analysis: To 10 mL of *Sample solution* in a test tube, add 1 mL of glacial acetic acid and 3 mL of a solution of potassium acetate (1 in 2). Shake the mixture vigorously, then gently rub the inner wall of the test tube with a glass rod for a few min, and allow to stand for 1 h.
Acceptance criteria: No white, crystalline precipitate soluble in 6 N ammonium hydroxide is formed.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed 1000 cfu/g, and the total combined molds and yeasts count does not exceed 100 cfu/g. It meets the requirements of the test for absence of *Escherichia coli*.
- **pH (791)**
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: 3.3–4.3

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, single-dose containers. Store at controlled room temperature.
- **LABELING:** The label specifies the directions for the constitution of the powder and states the equivalent amount of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).
- **USP REFERENCE STANDARDS (11)**
USP Citric Acid RS

Magnesium Carbonate and Sodium Bicarbonate for Oral Suspension

» Magnesium Carbonate and Sodium Bicarbonate for Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of $MgCO_3$ and $NaHCO_3$. It may contain suitable flavors.

Packaging and storage—Preserve in tight containers.

Identification—

A: Place about 1 g in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

B: The solution remaining in the flask responds to the tests for *Magnesium (191)* and for *Sodium (191)*.

Minimum fill (755): meets the requirements.

Acid-neutralizing capacity (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recom-

mended in the labeling, and not less than the number of mEq calculated by the formula:

$$0.8(0.024M) + 0.8(0.0119S)$$

in which 0.024 and 0.0119 are the theoretical acid-neutralizing capacities, in mEq, of MgCO_3 and NaHCO_3 , respectively; and M and S are the quantities, in mg, of MgCO_3 and NaHCO_3 in the specimen tested, based on the labeled quantities.

Assay for magnesium carbonate—Transfer an accurately weighed portion of Magnesium Carbonate and Sodium Bicarbonate for Oral Suspension, equivalent to about 4.2 g of MgCO_3 , to a 500-mL volumetric flask. Add 200 mL of 1 N hydrochloric acid, and mix. When dissolved, dilute with water to volume, and mix. Transfer 10.0 mL of this stock solution to a suitable container, dilute with water to 100 mL, add 10 mL of ammonia-ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium consumed is equivalent to 4.216 mg of MgCO_3 .

Assay for sodium bicarbonate—

Standard preparations—Dissolve a suitable quantity of sodium chloride, previously dried at 125° for 30 minutes and accurately weighed, in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 600 μg per mL. On the day of use, further dilute this solution quantitatively with water to obtain three solutions containing 6.0, 12.0, and 18.0 μg of sodium chloride per mL, respectively.

Assay preparation—Transfer an accurately measured volume of the stock solution remaining from the Assay for magnesium carbonate, equivalent to about 180 mg of NaHCO_3 , to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of the resulting solution to a 1000-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the Standard preparations and the Assay preparation at the sodium emission line at about 589.0 nm, with a suitable atomic absorption spectrophotometer (see Atomic Absorption Spectroscopy (852)) equipped with a sodium hollow-cathode lamp and an air-acetylene flame, using water as the blank. Plot the absorbances of the Standard preparations versus concentration, in μg of sodium chloride per mL, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, in μg per mL, of sodium chloride equivalent in the Assay preparation. Calculate the quantity, in g, of NaHCO_3 in the portion of Magnesium Carbonate and Sodium Bicarbonate for Oral Suspension taken by the formula:

$$(84.01 / 58.44)(C / V)$$

in which 84.01 and 58.44 are the molecular weights of sodium bicarbonate and sodium chloride, respectively; C is the concentration, in μg per mL, of sodium chloride equivalent in the Assay preparation; and V is the volume, in mL, of the stock solution remaining from the Assay for magnesium carbonate taken.

Magnesium Chloride

$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	203.30
Magnesium chloride, hexahydrate [7791-18-6].	
Anhydrous	95.21
[7786-30-3].	

DEFINITION

Magnesium Chloride contains NLT 98.0% and NMT 101.0% of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$.

IDENTIFICATION

A. IDENTIFICATION TESTS—GENERAL, Magnesium (191)

Sample solution: 50 mg/mL

B. IDENTIFICATION TESTS—GENERAL, Chloride (191)

Sample solution: 50 mg/mL

[NOTE—Acidify the Sample solution with diluted nitric acid before adding 6 N ammonium hydroxide.]

ASSAY

PROCEDURE

Sample: 450 mg

Analysis: Dissolve the Sample in 25 mL of water, add 5 mL of ammonia-ammonium chloride buffer TS and 0.1 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M disodium edetate is equivalent to 10.17 mg of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$.

Acceptance criteria: 98.0%–101.0%

IMPURITIES

INSOLUBLE MATTER

Sample: 20 g

Analysis: Dissolve the Sample in 200 mL of water, heat to boiling, and digest in a covered beaker on a steam bath for 1 h. Filter through a tared filtering crucible, wash thoroughly, dry at 115° , and determine the weight of the residue.

Acceptance criteria: NMT 0.005%

CHLORIDE AND SULFATE, Sulfate (221)

Sample: 10 g

Acceptance criteria: It shows no more sulfate than corresponds to 0.50 mL of 0.020 N sulfuric acid (0.005%).

BARIUM

Sample: 1 g

Analysis: Dissolve the Sample in 10 mL of water, and add 1 mL of 2 N sulfuric acid.

Acceptance criteria: No turbidity is produced within 2 h.

LIMIT OF CALCIUM

[NOTE—A commercially available atomic absorption standard solution for calcium may be used where preparation of a calcium standard stock solution is described below. Concentrations of the Standard solutions and the Sample solution may be modified to fit the linear or working range of the instrument.]

Dilute hydrochloric acid: Dilute 100 mL of hydrochloric acid with water to 1000 mL.

Lanthanum solution: To 58.65 g of lanthanum oxide add 400 mL of water, and add, gradually with stirring, 250 mL of hydrochloric acid. Stir until dissolved, and dilute with water to 1000 mL.

Standard solutions: Transfer 249.7 mg of calcium carbonate, previously dried at 300° for 3 h and cooled in a desiccator for 2 h, to a 100-mL volumetric flask. Dissolve in a minimum amount of hydrochloric acid, and dilute with water to volume. Transfer 1.0, 5.0, 10.0, and 15.0 mL of this stock solution to separate 1000-mL volumetric flasks, each containing 20 mL of Lanthanum solution and 40 mL of Dilute hydrochloric acid. Dilute with water to volume. These Standard solutions contain 1.0, 5.0, 10.0, and 15.0 μg /mL of calcium, respectively.

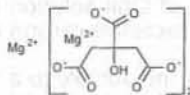
Blank solution: Transfer 4 mL of Lanthanum solution and 10 mL of Dilute hydrochloric acid to a 200-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 10.0 g of Magnesium Chloride to a 200-mL volumetric flask, and add water to dissolve. Add 4 mL of Lanthanum solution, and dilute with water to volume.

Instrumental conditions(See *Atomic Absorption Spectroscopy* (852).)**Mode:** Atomic absorption spectrophotometry**Lamp:** Calcium hollow-cathode**Flame:** Nitrous oxide-acetylene**Analytical wavelength:** Calcium emission line at 422.7 nm**Analysis****Samples:** *Standard solutions*, *Blank solution*, and *Sample solution*.Determine the concentration, *C*, in µg/mL, of calcium in the *Sample solution* using the calibration graph.

Calculate the percentage of calcium in the portion of Magnesium Chloride taken:

$$\text{Result} = (V/W \times C \times F) \times 100$$

V = volume of the *Sample solution* (mL)*W* = weight of Magnesium Chloride taken (mg)*C* = as defined above*F* = conversion factor from µg/mL to mg/mL, 0.001**Acceptance criteria:** NMT 0.01%**POTASSIUM****Sample solution:** 5 g**Analysis:** Dissolve the *Sample* in 5 mL of water, and add 0.2 mL of sodium bitartrate TS.**Acceptance criteria:** No turbidity is produced within 5 min.**ALUMINUM (206)** (where it is labeled as intended for use in hemodialysis)**Test preparation:** Prepare as directed in the chapter, using 2.0 g.**Acceptance criteria:** NMT 1 ppm**Delete the following:****HEAVY METALS (231)****Test preparation:** Dissolve 2 g in water, and dilute with water to 25 mL.**Acceptance criteria:** NMT 10 ppm • (Official 1-Jan-2018)**SPECIFIC TESTS****pH (791)****Sample solution:** 50 mg/mL in carbon dioxide-free water**Acceptance criteria:** 4.5–7.0**ADDITIONAL REQUIREMENTS****PACKAGING AND STORAGE:** Preserve in tight containers.**LABELING:** Where Magnesium Chloride is intended for use in hemodialysis, it is so labeled.**Magnesium Citrate** $C_{12}H_{10}Mg_3O_{14}$ 451.11

1,2,3-Propanetricarboxylic acid, hydroxy-, magnesium salt (2:3);

Magnesium citrate (3:2) [3344-18-1].

DEFINITION

Magnesium Citrate contains NLT 14.5% and NMT 16.4% of magnesium (Mg), calculated on the dried basis.

IDENTIFICATION**A. IDENTIFICATION TESTS—GENERAL, Magnesium (191)****Sample:** 10 mg/mL**Acceptance criteria:** Meets the requirements**B. IDENTIFICATION TESTS—GENERAL, Citrate (191)****Sample:** 80 mg/mL**Acceptance criteria:** Meets the requirements**ASSAY****PROCEDURE****Sample:** 400 mg**Analysis:** Dissolve the *Sample* in 50 mL of water. Add 20 mL of ammonia-ammonium chloride buffer TS and 0.1 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination (see *Titrimetry* (541)), and make any necessary correction. From the volume of 0.05 M edetate disodium consumed, deduct the volume of 0.05 M edetate disodium corresponding to the amount of calcium in the portion of Magnesium Citrate taken, based on the amount of calcium found in the test for *Limit of Calcium*. Each mg of Ca is equivalent to 0.25 mL of 0.05 M edetate disodium. The difference is the volume of 0.05 M edetate disodium consumed by the magnesium. Each mL of 0.05 M edetate disodium is equivalent to 1.215 mg of Mg.**Acceptance criteria:** 14.5%–16.4% on the dried basis**IMPURITIES****CHLORIDE AND SULFATE, Chloride (221)****Sample:** 300 mg**Acceptance criteria:** It shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.05%).**CHLORIDE AND SULFATE, Sulfate (221)****Sample:** 100 mg**Acceptance criteria:** It shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.2%).**ARSENIC, Method I (211):** NMT 3 ppm**Delete the following:****HEAVY METALS, Method I (231)****Test preparation:** Dissolve 0.4 g in 25 mL of water, and proceed as directed in the chapter, except use glacial acetic acid to adjust the pH.**Acceptance criteria:** NMT 50 ppm • (Official 1-Jan-2018)**IRON (241)****Test preparation:** Boil 50 mg with 5 mL of 2 N nitric acid for 1 min. Cool, dilute with water to 45 mL, and add 2 mL of hydrochloric acid.**Acceptance criteria:** NMT 200 ppm**LIMIT OF CALCIUM**[NOTE—A commercially available atomic absorption standard solution for calcium may be used where preparation of a calcium standard stock solution is described below. Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the instrument.]**Dilute hydrochloric acid:** Dilute 100 mL of hydrochloric acid with water to 1000 mL.**Lanthanum solution:** To 58.65 g of lanthanum oxide add 400 mL of water, and add, gradually with stirring, 250 mL of hydrochloric acid. Stir until dissolved, and dilute with water to 1000 mL.**Standard solutions:** Transfer 249.7 mg of calcium carbonate, previously dried at 300° for 3 h and cooled in a desiccator for 2 h, to a 100-mL volumetric flask. Dissolve in a minimum amount of hydrochloric acid, and dilute with water to volume. Transfer 1.0, 5.0, 10.0, and 15.0 mL of this stock solution to separate 1000-mL volumetric flasks, each containing 20 mL of *Lanthanum solution* and 40 mL of *Dilute hydrochloric acid*. Dilute

with water to volume. These *Standard solutions* contain 1.0, 5.0, 10.0, and 15.0 µg/mL of calcium, respectively.

Sample solution: Transfer 250 mg of Magnesium Citrate to a beaker, add 30 mL of *Dilute hydrochloric acid*, and stir until dissolved. Transfer the solution to a 200-mL volumetric flask containing 4 mL of *Lanthanum solution*, and dilute with water to volume.

Blank solution: Transfer 4 mL of *Lanthanum solution* and 10 mL of *Dilute hydrochloric acid* to a 200-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Nitrous oxide-acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank solution*

Determine the concentration, *C*, in µg/mL, of calcium in the *Sample solution* using the calibration graph.

Calculate the percentage of calcium in the portion of Magnesium Citrate taken:

$$\text{Result} = (V/W \times C \times F) \times 100$$

V = volume of the *Sample solution* (mL)

W = weight of Magnesium Citrate taken (mg)

C = concentration of calcium in the *Sample solution* (µg/mL)

F = conversion from µg/mL to mg/mL, 0.001

Acceptance criteria: NMT 1.0% on the dried basis

SPECIFIC TESTS

• **pH (791):** 5.0–9.0, in a suspension (50 mg/mL)

• **LOSS ON DRYING (731)** Dry 1 g in a mechanical convection oven at 135° for 16 h, then to constant weight: it loses NMT 29% of its weight, except that where it is labeled as anhydrous, it loses NMT 2.0% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Magnesium Citrate that loses NMT 2.0% of its weight in the test for *Loss on Drying* may be labeled as Anhydrous Magnesium Citrate.

Magnesium Citrate Oral Solution

DEFINITION

Magnesium Citrate Oral Solution is a sterilized or pasteurized solution containing NLT 7.59 g of anhydrous citric acid (C₆H₈O₇) and an amount of magnesium citrate equivalent to NLT 1.55 g and NMT 1.9 g of magnesium oxide (MgO) in each 100 mL of Oral Solution.

Prepare Magnesium Citrate Oral Solution as follows.

Magnesium Carbonate	15 g
Anhydrous Citric Acid	27.4 g
Syrup	60 mL
Talc	5 g
Lemon Oil	0.1 mL
Potassium Bicarbonate	2.5 g
Purified Water, a sufficient quantity to make	350 mL

Dissolve the *Anhydrous Citric Acid* in 150 mL of hot *Purified Water* in a suitable dish, slowly add the *Magnesium Carbonate* previously mixed with 100 mL of *Purified Water*, and stir until it is dissolved. Add the *Syrup*, heat the mixed liquids to the boiling point, and immediately add the *Lemon Oil* previously triturated with *Talc*. Filter the

mixture, while hot, into a strong bottle of suitable capacity previously rinsed with boiling *Purified Water*. Add boiled *Purified Water* to bring the preparation to final volume. Use *Purified Cotton* as a stopper for the bottle, and allow to cool. Add the *Potassium Bicarbonate*, and immediately insert the stopper in the bottle securely. Shake the solution occasionally until the *Potassium Bicarbonate* is dissolved, cap the bottle, and sterilize or pasteurize the solution.

Alternatively, 30 g of citric acid containing 1 molecule of water of hydration, equivalent to 27.4 g of *Anhydrous Citric Acid*, may be used in the foregoing formula. In this process, replace the 2.5 g of *Potassium Bicarbonate* with 2.1 g of sodium bicarbonate, preferably in tablet form. The Oral Solution may be further carbonated by the use of carbon dioxide under pressure.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL, Magnesium (191):**

Meets the requirements

• **B.**

Sample: 5 mL

Analysis: To the *Sample* add 1 mL of potassium permanganate TS and 5 mL of mercuric sulfate TS, and heat the solution.

Acceptance criteria: A white precipitate is formed.

ASSAY

• **CITRIC ACID**

Mobile phase, Standard preparation 1, and Chromatographic system: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345).

Assay preparation: Transfer 10 mL of Oral Solution that has been previously freed from excessive carbon dioxide by repeated pouring to a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345), *Assay Preparation for Citric Acid/Citrate and Phosphate*.

Analysis: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345), *Procedure*.

Calculate the quantity, *g*, of anhydrous citric acid (C₆H₈O₇) in 100 mL of Oral Solution:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (M_{r1}/M_{r2}) \times F$$

r_U = peak area of citrate from the *Assay preparation*

r_S = peak area of citrate from *Standard preparation*

C_S = concentration of citrate in *Standard preparation 1* (µg/mL)

V = final volume of Oral Solution, 100 mL

D = dilution factor

M_{r1} = molecular weight of citric acid (C₆H₈O₇), 192.12

M_{r2} = molecular weight of citrate (C₆H₅O₇), 189.10

F = conversion factor, 10⁻⁶ g/µg

Acceptance criteria: NLT 7.59 g in 100 mL of Oral Solution

• **MAGNESIUM OXIDE**

Sample: 50.0 mL of Oral Solution that has been previously freed from excessive carbon dioxide by repeated pouring

Analysis: Transfer the *Sample* to a 100-mL volumetric flask, and dilute with water to volume. Transfer 5.0 mL of the resulting solution to a beaker containing 150 mL of water heated to 70°–80°, and add 1 mL of ammonium chloride TS and 3 mL of ammonium hydroxide. Mix, and slowly add 8 mL of 8-hydroxyquinoline TS with stirring. After standing for 30 min, filter through a sintered-glass crucible, previously dried and weighed, and wash the precipitate with ten 10-mL portions of water. Dry the crucible and contents at 105° for 3 h, cool, and weigh. Determine the equivalent of magnesium oxide (MgO) in 100 mL of Oral Solution by multi-

plying the weight of $C_{18}H_{12}MgN_2O_2 \cdot 2H_2O$ so obtained by 4.624.

Acceptance criteria: 1.55–1.9 g in 100 mL of Oral Solution

IMPURITIES

- **CHLORIDE AND SULFATE, Chloride** (221)
Sample: 2.0 mL
Acceptance criteria: 0.01%; it shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid.
- **CHLORIDE AND SULFATE, Sulfate** (221)
Sample: 2.0 mL
Acceptance criteria: 0.015%; it shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid.
- **TARTARIC ACID**
Sample: 10 mL
Analysis: Place the *Sample* in a test tube, add 1 mL of glacial acetic acid and 3 mL of a solution of potassium acetate (1 in 2), shake the mixture vigorously, then gently rub the inner wall of the test tube with a glass rod for a few min, and allow to stand for 1 h.
Acceptance criteria: No white, crystalline precipitate soluble in 6 N ammonium hydroxide is formed.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in bottles NLT 200 mL in capacity. Store at controlled room temperature or in a cool place.
- **USP REFERENCE STANDARDS** (11)
USP Citric Acid RS

Magnesium Citrate for Oral Solution

DEFINITION

Magnesium Citrate for Oral Solution, when constituted as directed in the labeling, yields a solution that contains NLT 90.0% and NMT 110.0% of the labeled amount of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Magnesium** (191)
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: Meets the requirements
- **B.** The retention time of the citrate peak of the *Sample solution* corresponds to that of *Standard solution 1*, as obtained in the test for *Content of Anhydrous Citric Acid*.

ASSAY

• PROCEDURE

Sample solution: Transfer a volume of constituted oral solution, equivalent to 18.7 g of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$), to a 1000-mL volumetric flask. Add 200 mL of 1 N hydrochloric acid, swirl, and allow to stand for 10 min. Dilute with water to volume. Stir by mechanical means for about 30 min.

Analysis: Transfer 10.0 mL of the *Sample solution* to a 250-mL beaker. Add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS until the last hint of violet disappears (blue endpoint). Each mL of 0.05 M edetate disodium is equivalent to 7.520 mg of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$).

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• CONTENT OF ANHYDROUS CITRIC ACID

Mobile phase, Standard solution 1, Chromatographic system, and System suitability: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345).

Sample solution: Transfer an appropriate volume of the constituted oral solution into a suitable volumetric flask, and proceed as directed for the *Sample solution* in *Assay for Citric Acid/Citrate and Phosphate* (345), *Assay Preparation for Citric Acid/Citrate Assay*.

Analysis

Samples: *Standard solution 1* and *Sample solution* Proceed as directed for *Assay for Citric Acid/Citrate and Phosphate* (345), *Procedure*.

Calculate the percentage of anhydrous citric acid ($C_6H_8O_7$) in relation to the labeled amount of magnesium citrate in the volume of constituted oral solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of citrate from the *Sample solution*

r_S = peak area of citrate from *Standard solution 1*

C_S = concentration of citrate in *Standard solution 1* (mg/mL)

C_U = nominal concentration of magnesium citrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of anhydrous citric acid, 192.12

M_{r2} = molecular weight of citrate ($C_6H_5O_7$), 189.10

Acceptance criteria: 76.6%–93.7% of the labeled amount of magnesium citrate

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

IMPURITIES

- **CHLORIDE AND SULFATE, Chloride** (221)
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: A 2.0-mL portion of *Sample solution* shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid (NMT 0.01%).
- **CHLORIDE AND SULFATE, Sulfate** (221)
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: A 2.0-mL portion of *Sample solution* shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid (NMT 0.015%).
- **TARTARIC ACID**
Sample solution: Constitute as directed in the labeling.
Analysis: To 10 mL of *Sample solution* in a test tube, add 1 mL of glacial acetic acid and 3 mL of a solution of potassium acetate (1 in 2). Shake the mixture vigorously, then gently rub the inner wall of the test tube with a glass rod for a few min, and allow to stand for 1 h.
Acceptance criteria: No white, crystalline precipitate soluble in 6 N ammonium hydroxide is formed.

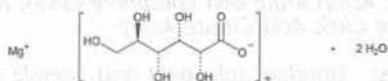
SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the test for absence of *Escherichia coli* and *Salmonella* species.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The label contains directions for constitution of the powder and states the equivalent amount of magnesium citrate ($C_{12}H_{10}Mg_3O_{14}$) in a given volume of the oral solution obtained after constitution.
- **USP REFERENCE STANDARDS** (11)
USP Citric Acid RS

Magnesium Gluconate



$C_{12}H_{22}MgO_{14}$ 414.60

$C_{12}H_{22}MgO_{14} \cdot 2H_2O$ 450.64

D-Gluconic acid, magnesium salt (2:1), hydrate;
Magnesium D-gluconate (1:2) hydrate;
Magnesium D-gluconate (1:2) dihydrate [59625-89-7].
Anhydrous [3632-91-5].

DEFINITION

Magnesium Gluconate contains NLT 98.0% and NMT 102.0% of anhydrous magnesium gluconate ($C_{12}H_{22}MgO_{14}$), calculated on the anhydrous basis.

IDENTIFICATION

- A. IDENTIFICATION TESTS—GENERAL, Magnesium (191):** A 100-mg/mL solution meets the requirements.

- B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: 10 mg/mL of Magnesium Gluconate, heating in a water bath at 60°, if necessary, to dissolve

Chromatographic system
(See Chromatography (621), Thin-Layer Chromatography.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 μ L

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: Standard solution and Sample solution

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the Spray reagent. Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the Sample solution corresponds in color, size, and R_f value to that of the Standard solution.

ASSAY

PROCEDURE

Sample: 800 mg of Magnesium Gluconate

Blank: 20 mL of water

Titrimetric system

(See Titrimetry (541).)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Dissolve the Sample in 20 mL of water. Add 5 mL of ammonia-ammonium chloride buffer TS and 0.1 mL of eriochrome black TS. Titrate with Titrant to a blue endpoint. Perform the Blank determination. Calculate the percentage of magnesium gluconate ($C_{12}H_{22}MgO_{14}$) in the Sample taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F] / W\} \times 100\}$$

V_S = Titrant volume consumed by the Sample (mL)

V_B = Titrant volume consumed by the Blank (mL)

M = Titrant molarity (mM/mL)

F = equivalency factor, 414.6 mg/mM

W = Sample weight (mg)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- CHLORIDE AND SULFATE, Chloride (221)**

Standard solution: 0.7 mL of 0.020 N hydrochloric acid

Sample: 1.0 g

Acceptance criteria: NMT 0.05%

- CHLORIDE AND SULFATE, Sulfate (221)**

Standard solution: 1.0 mL of 0.020 N sulfuric acid

Sample: 2.0 g

Acceptance criteria: NMT 0.05%

Delete the following:

- HEAVY METALS (231)**

Test preparation: 1.0 g in 10 mL of water. Add 6 mL of 3 N hydrochloric acid, and dilute with water to 25 mL.

Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

- REDUCING SUBSTANCES**

Sample: 1.0 g of Magnesium Gluconate

Blank: 10 mL of water

Titrimetric system

(See Titrimetry (541).)

Mode: Residual titration

Titrant: 0.1 N iodine VS

Back titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the Sample to a 250-mL conical flask, dissolve in 10 mL of water, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of Titrant, and 10 mL of 3 N hydrochloric acid, and titrate with Back titrant, adding 3 mL of starch TS as the endpoint is approached. Perform the Blank determination.

Calculate the percentage of reducing substances (as dextrose) in the Sample taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F] / W\} \times 100$$

V_B = Back titrant volume consumed by the Blank (mL)

V_S = Back titrant volume consumed by the Sample (mL)

N = Back titrant normality (mEq/mL)

F = equivalency factor, 27 mg/mEq

W = Sample weight (mg)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

- PH (791)**

Sample solution: 50 mg/mL

Acceptance criteria: 6.0–7.8

- WATER DETERMINATION, Method 1b (921)**

Test preparation: Proceed as directed in the chapter. Allow 30 min for solubilization of Magnesium Gluconate and for the reaction to reach completion.

Blank: Use the same volume of Reagent as that of the Test preparation but without the specimen.

Analysis

Samples: Test preparation and Blank

Calculate the water content, in mg, of the Magnesium Gluconate taken:

$$\text{Result} = F \times (X_B - X) \times R$$

X_B = volume of standardized Water-Methanol Solution required to neutralize the unconsumed Reagent in the Blank determination (mL)

The other terms are as defined in Water Determination, Method 1b (921).

Acceptance criteria: 3.0%–12.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
USP Potassium Gluconate RS

Magnesium Gluconate Tablets

DEFINITION

Magnesium Gluconate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of magnesium gluconate ($C_{12}H_{22}MgO_{14}$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Magnesium (191)**
Sample solution: A filtered solution in water from powdered Tablets, equivalent to 100 mg/mL of magnesium gluconate
Acceptance criteria: Meet the requirements
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**
Standard solution: 10 mg/mL of USP Potassium Gluconate RS
Sample solution: Equivalent to 10 mg/mL of magnesium gluconate from a dilution of the *Sample solution* obtained for the *Identification test A*
Chromatographic system
(See *Chromatography (621)*, *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel
Application volume: 5 μ L
Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)
Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.
Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_f value to that of the *Standard solution*.

ASSAY

• PROCEDURE

Sample: A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 800 mg of magnesium gluconate
Blank: Proceed as directed in the *Analysis* without the *Sample*.
Titrimetric system
(See *Titrimetry (541)*.)
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a crucible, and ignite, gently at first, until free from carbon. Cool the crucible, add 25 mL of water and 5 mL of hydrochloric acid, and stir. Heat on a steam bath for 5 min. Filter, rinsing the filter with several portions of water. Dilute the combined filtrate and washings with water to 150 mL. Add ammonia–ammonium chloride buffer TS until the solution is neutral to litmus. Add an excess of 5 mL of am-

monia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS, and titrate with *Titrant* to a blue endpoint. Perform a *Blank* determination.

Calculate the percentage of the labeled amount of magnesium gluconate ($C_{12}H_{22}MgO_{14}$) in the portion of Tablets taken:

$$\text{Result} = \frac{[(V_S - V_B) \times M \times F/W]}{100} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
 V_B = *Titrant* volume consumed by the *Blank* (mL)
 M = actual molarity of the *Titrant* (mM/mL)
 F = equivalency factor, 414.6 mg/mM
 W = nominal amount of magnesium gluconate in the *Sample* taken (mg)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: Solution having a known concentration of magnesium in the *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

Instrumental conditions

(See *Atomic Absorption Spectroscopy (852)*.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 285.2 nm

Lamp: Magnesium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solution* and *Sample solution*
Determine the concentration of magnesium (Mg) in the *Sample solution* in comparison with a *Standard solution*. Calculate the percentage of the labeled amount of magnesium gluconate ($C_{12}H_{22}MgO_{14}$) dissolved:

$$\text{Result} = \frac{(C \times D \times V/L) \times (M_r/A_r)}{100} \times 100$$

C = determined concentration of magnesium in the *Sample solution* (mg/mL)

D = dilution factor for the *Sample solution*

V = volume of *Medium*, 900 mL

L = label amount of magnesium gluconate (mg/Tablet)

M_r = molecular weight of magnesium gluconate, 414.60

A_r = atomic weight of magnesium, 24.31

Tolerances: NLT 80.0% (Q) of the labeled amount of magnesium gluconate ($C_{12}H_{22}MgO_{14}$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
USP Potassium Gluconate RS

Magnesium Hydroxide

$Mg(OH)_2$ 58.32

Magnesium hydroxide.

Magnesium hydroxide [1309-42-8].

» Magnesium Hydroxide, dried at 105° for 2 hours, contains not less than 95.0 percent and not more than 100.5 percent of $Mg(OH)_2$.

Packaging and storage—Preserve in tight containers.

Identification—A 1 in 20 solution in 3 N hydrochloric acid responds to the tests for *Magnesium* (191).

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the test for absence of *Escherichia coli*.

Loss on drying (731)—Dry it at 105° for 2 hours: it loses not more than 2.0% of its weight.

Loss on ignition (733)—Ignite it at 800°, increasing the heat gradually, to constant weight: it loses between 30.0% and 33.0% of its weight.

Soluble salts—Boil 2.0 g with 100 mL of water for 5 minutes in a covered beaker, filter while hot, cool, and dilute the filtrate with water to 100 mL. Titrate 50 mL of the diluted filtrate with 0.10 N sulfuric acid, using methyl red TS as the indicator: not more than 2.0 mL of the acid is consumed. Evaporate 25 mL of the diluted filtrate to dryness, and dry at 105° for 3 hours: not more than 10 mg of residue remains.

Carbonate—Boil a mixture of 0.10 g with 5 mL of water, cool, and add 5 mL of 6 N acetic acid: not more than a slight effervescence is observed.

Limit of calcium—

Dilute hydrochloric acid, Lanthanum solution, Standard preparations, and Blank solution—Prepare as directed in the test for Limit of calcium under *Magnesium Carbonate*.

Test preparation—Transfer 250 mg of Magnesium Hydroxide, previously dried, to a beaker, add 30 mL of Dilute hydrochloric acid, and stir until dissolved, heating if necessary. Transfer the solution so obtained to a 200-mL volumetric flask containing 4 mL of Lanthanum solution, dilute with water to volume, and mix.

Procedure—Proceed as directed in the test for Limit of calcium under *Magnesium Carbonate*: the limit is 1.5%.

Delete the following:

***Heavy metals, Method 1** (231)—Dissolve 1.0 g in 15 mL of 3 N hydrochloric acid, and evaporate the solution on a steam bath to dryness. Toward the end of the evaporation, stir the residue frequently, disintegrate it so that finally a dry powder is obtained, dissolve the residue in 20 mL of water, and filter. To the filtrate, which should be neutral to litmus, add 2 mL of 1 N acetic acid, and dilute with water to 25 mL: the limit is 20 µg per g. (Official 1-Jan-2018)

Limit of lead—[NOTE—When water is specified as a diluent, use deionized ultra-filtered water.]

Blank solution—Transfer 3.0 mL of nitric acid to a 50-mL volumetric flask, and dilute with water to volume.

Thallium internal standard 20 ppb—[NOTE—Use this solution only if an ICP-MS instrument is used. This internal standard is added in-line via a mixing block between the sample probe and the spray chamber.] Dilute 20.0 mL of a commercially prepared thallium ICP standard solution (1000 ppb) with water to 1 L.

Dilute nitric acid—Dilute 2.0 mL of nitric acid with water to 100 mL.

Standard stock solution 100 ppb—Prepare this solution fresh every two months. Quantitatively dilute an accurately measured volume of a commercially prepared lead ICP standard (1000 ppm) with Dilute nitric acid to obtain a solution containing 10 ppm of lead. Further dilute this solution with Dilute nitric acid to obtain a solution containing 1000 ppb of lead. Transfer 10.0 mL of this solution to a separate 100-mL volumetric flask, add 2.0 mL of nitric acid, and dilute with water to volume.

Standard solutions—Prepare these solutions fresh weekly. [NOTE—The concentrations specified below are recommended if an ICP-MS instrument is used. If an ICP-AES instrument is used, the concentrations of the Standard solu-

tions may be modified to adapt to the working range of the instrument.] Transfer 5.0 mL of the Standard stock solution 100 ppb to a 50-mL volumetric flask, add 3.0 mL of nitric acid, and dilute with water to volume (Standard lead solution 10 ppb). Transfer 5.0 mL of Standard lead solution 10 ppb to a 50-mL volumetric flask, add 3.0 mL of nitric acid, and dilute with water to volume (Standard lead solution 1 ppb).

Test solution—[NOTE—The concentration specified below is recommended if an ICP-MS instrument is used. If an ICP-AES instrument is used, the concentration of the Test solution may be modified to adapt to the working range of the instrument.]

Accurately weigh about 0.25 g of Magnesium Hydroxide. Cautiously add 3.0 mL of nitric acid, and mix until the sample is dissolved. Accurately transfer this solution to a 50-mL volumetric flask, and dilute with water to volume.

Procedure (see *Plasma Spectrochemistry* (730))—The inductively coupled plasma-mass spectrometer (ICP-MS) is equipped with a quadrupole mass spectrometer and an ion detector maintained under vacuum. The instrument should read all isotopes for lead (206, 207, and 208 amu) and the thallium internal standard (205 amu), and should report the total lead content using the most naturally abundant isotope at 208 amu. Alternatively, lead could be determined using an inductively coupled plasma-atomic emission spectrometer (ICP-AES) by measuring the emission at 220.353 nm, with the settings optimized as directed by the manufacturer. [NOTE—To minimize matrix interference when using an ICP-AES instrument, it is recommended that the method of standard additions be used.]

Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check. Generate the calibration curve using the Blank solution, Standard lead solution 1 ppb, and Standard lead solution 10 ppb: a linear regression coefficient is not less than 0.999.

Aspirate the Test solution, at least in duplicate, and calculate the amount of lead using the calibration curve. Report the average reading as the lead content of the sample. Calculate the content of lead in the portion of Magnesium Hydroxide taken: not more than 0.00015% (1.5 ppm) is found.

Assay—Transfer about 75 mg of Magnesium Hydroxide, previously dried and accurately weighed, to a conical flask. Add 2 mL of 3 N hydrochloric acid, and swirl to dissolve. Add 100 mL of water, adjust the reaction of the solution to a pH of 7 (using pH indicator paper; see *Indicator and Test Papers* under *Reagents* in the section *Reagents, Indicators, and Solutions*) with 1 N sodium hydroxide, add 5 mL of ammonia-ammonium chloride buffer TS and 0.15 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of Mg(OH)₂.

Magnesium Hydroxide Paste

» Magnesium Hydroxide Paste is an aqueous paste of Magnesium Hydroxide. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of magnesium hydroxide [Mg(OH)₂], the labeled amount being not less than 28.0 percent and not more than 70.0 percent of magnesium hydroxide.

Packaging and storage—Preserve in tight containers.

Identification—One g of Paste dissolved in 10 mL of 3 N hydrochloric acid responds to the tests for *Magnesium* (191).

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—Its total aerobic microbial count does not exceed 400 cfu per g, and it meets the requirements of the test for absence of *Escherichia coli*.

Change to read:

Soluble alkalies—Accurately weigh a portion of Paste, equivalent to about 7.75 g of magnesium hydroxide, and mix with 75.0 mL of water. Transfer about 25 mL of this diluted Paste to a filter, and reject the first 5 mL of the filtrate. [NOTE—Retain the remaining diluted Paste for the test for *Carbonate and acid-insoluble matter*.] (Official 1-Jan-2018) Dilute 5 mL of the clear filtrate with 40 mL of water. Add 1 drop of methyl red TS, and titrate the solution with 0.10 N sulfuric acid to the production of a persistent pink color: not more than 1.0 mL of the acid is required.

Soluble salts—To 5.0 mL of the clear filtrate obtained in the test for *Soluble alkalies* add 3 drops of sulfuric acid, evaporate on a steam bath to dryness, and ignite gently to constant weight: the residue weighs not more than 12 mg.

Carbonate and acid-insoluble matter—To 1 mL of the diluted Paste obtained in the test for *Soluble alkalies* add 2 mL of 3 N hydrochloric acid: not more than a slight effervescence occurs, and the solution is not more than slightly turbid.

Limit of calcium—

Dilute hydrochloric acid, Lanthanum solution, Standard preparations, and Blank solution—Prepare as directed in the test for *Limit of calcium* under *Magnesium Carbonate*.

Test preparation—Transfer a portion of the Paste, equivalent to 250 mg of $Mg(OH)_2$, to a beaker, add 30 mL of *Dilute hydrochloric acid*, and stir until dissolved, heating if necessary. Transfer the solution so obtained to a 200-mL volumetric flask containing 4 mL of *Lanthanum solution*, dilute with water to volume, and mix.

Procedure—Proceed as directed in the test for *Limit of calcium* under *Magnesium Carbonate*: the limit is 1.5%.

Delete the following:

• **Heavy metals, Method I (231)**—To 4.0 mL of the diluted Paste obtained in the test for *Soluble alkalies* add 6 mL of 3 N hydrochloric acid, and evaporate the solution on a steam bath to dryness, with frequent stirring. Dissolve the residue in 20 mL of water, and evaporate to dryness in the same manner as before. Redissolve in 20 mL of water, filter if necessary, and dilute with water to 25 mL: the limit is 5 ppm, based on the amount of diluted Paste taken. (Official 1-Jan-2018)

Limit of lead—

Blank solution, Thallium internal standard 20 ppb, Dilute nitric acid, Standard stock solution 100 ppb, and Standard solutions—Proceed as directed in the test for *Limit of lead* under *Magnesium Hydroxide*.

Test solution—Accurately weigh an amount of Paste equivalent to 0.25 g of magnesium hydroxide. Cautiously add 3.0 mL of nitric acid, and mix until the sample is dissolved. Accurately transfer this solution to a 50-mL volumetric flask, and dilute with water to volume. [NOTE—This concentration is recommended if an ICP-MS instrument is used. If an ICP-AES instrument is used, the concentration of the *Test solution* may be modified to adapt to the working range of the instrument.]

Procedure—Proceed as directed in the test for *Limit of lead* under *Magnesium Hydroxide*. Calculate the content of lead in the portion of Paste taken based on the content of magnesium hydroxide in the Paste, as determined in the *Assay*: not more than 0.00015% (1.5 ppm) is found.

Assay—Transfer an accurately weighed portion of Paste, equivalent to about 250 mg of magnesium hydroxide, to a 100-mL volumetric flask. Dissolve in 10 mL of 3 N hydrochloric acid, dilute with water to volume, and mix. Filter, if necessary, and transfer 25.0 mL of the filtrate to a beaker containing 75 mL of water, and mix. Adjust the reaction of the solution to a pH of 7 (using pH indicator paper; see *Indicator and Test Papers* under *Reagents* in the section *Reagents, Indicators, and Solutions*) with 1 N sodium hydroxide, add 5 mL of ammonia-ammonium chloride buffer TS and 0.15 mL of eriochrome black TS, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of magnesium hydroxide [$Mg(OH)_2$].

Magnesium Oxide

MgO 40.30
Magnesium oxide [1309-48-4].

DEFINITION

Magnesium Oxide, after ignition, contains NLT 96.0% and NMT 100.5% of magnesium oxide (MgO).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Magnesium (191):** A solution in diluted hydrochloric acid meets the requirements.

ASSAY

• PROCEDURE

Diluted ammonium hydroxide: Dilute 67 g of ammonium hydroxide (about 75 mL) with water to 100 mL.

Buffer: Prepare ammonium chloride buffer pH 10 as follows. In a 100-mL volumetric flask, dissolve 5.4 g of ammonium chloride in 20 mL of water, add 35 mL of *Diluted ammonium hydroxide*, and dilute with water to volume.

Sample stock solution: Ignite a sample of Magnesium Oxide to a constant weight in the temperature range of $(800^{\circ}\text{--}900^{\circ}) \pm 25^{\circ}$. Weigh 320 mg of the ignited sample into a 100-mL volumetric flask, dissolve in 20 mL of 2 N hydrochloric acid, and dilute with water to volume.

Sample solution: Transfer 20.0 mL of the *Sample stock solution* to a 500-mL flask, and dilute with water to 300 mL. The *Sample solution* is equivalent to 64 mg of ignited Magnesium Oxide.

Analysis: To the *Sample solution*, add 10 mL of *Buffer* and 50 mg of eriochrome black T-sodium chloride indicator. Heat the sample to 40° , and titrate to a blue endpoint with 0.1 M edetate disodium VS. Perform a blank determination, and make any necessary correction to determine the volume of 0.1 M edetate disodium consumed (V_s).

Calculate the volume of 0.1 M edetate disodium, V_{Ca} , in mL, consumed by calcium, which is present in the portion of Magnesium Oxide taken:

$$V_{Ca} = (W \times L_{Ca}) / (F_{Ca} \times 100)$$

W = amount of ignited Magnesium Oxide in the *Sample solution* (mg)

L_{Ca} = content of calcium as determined in the test for *Limit of Calcium* (%)

F_{Ca} = weight of calcium equivalent to each mL of 0.1 M edetate disodium, 4.008 mg

Calculate the percentage of magnesium oxide (MgO) in the portion of Magnesium Oxide taken:

$$\text{Result} = (V_s - V_{Ca}) \times (F_{MgO}/W) \times 100$$

V_s = volume of 0.1 M edetate disodium consumed by the *Sample solution* (mL)

- V_{Ca} = volume of 0.1 M edetate disodium consumed by calcium (mL)
 F_{MgO} = weight of Magnesium Oxide equivalent to each mL of 0.1 M edetate disodium, 4.030 mg
 W = amount of the ignited Magnesium Oxide in the *Sample solution* (mg)
Acceptance criteria: 96.0%–100.5% after ignition

IMPURITIES• **FREE ALKALI AND SOLUBLE SALTS**

Sample solution: Boil 2.0 g with 100 mL of water for 5 min in a covered beaker, and filter while hot. Allow to cool, and dilute with water to 100 mL.

Analysis 1: To 50 mL of the *Sample solution* add methyl red TS, and titrate with 0.10 N sulfuric acid.

Acceptance criteria 1: NMT 2.0 mL of the acid is consumed.

Analysis 2: Evaporate 25 mL of the remaining *Sample solution* to dryness, and dry at 105° for 1 h.

Acceptance criteria 2: NMT 10 mg of residue remains (NMT 2.0%).

• **ACID-INSOLUBLE SUBSTANCES**

Sample: 2 g

Analysis: Mix the *Sample* with 75 mL of water, add hydrochloric acid in small portions with agitation until no more dissolves, and boil for 5 min. If an insoluble residue remains, filter, wash well with water until the last washing is free from chloride, and ignite.

Acceptance criteria: The weight of the ignited residue is NMT 2 mg (NMT 0.1%).

• **LIMIT OF CALCIUM**

[NOTE—A commercially available atomic absorption standard solution for calcium may be used where preparation of a calcium standard stock solution is described below. Concentrations of the *Standard solutions* and the *Sample solution* may be modified to fit the linear or working range of the instrument.]

Dilute hydrochloric acid: Dilute 100 mL of hydrochloric acid with water to 1000 mL.

Lanthanum solution: To 58.65 g of lanthanum oxide, add 400 mL of water, and add, gradually with stirring, 250 mL of hydrochloric acid. Stir until dissolved, and dilute with water to 1000 mL.

Standard solutions: Transfer 249.7 mg of calcium carbonate, previously dried at 300° for 3 h and cooled in a desiccator for 2 h, to a 100-mL volumetric flask. Dissolve in a minimum amount of hydrochloric acid, and dilute with water to volume. Transfer 1.0, 5.0, 10.0, and 15.0 mL of this stock solution to separate 1000-mL volumetric flasks, each containing 20 mL of the *Lanthanum solution* and 40 mL of *Dilute hydrochloric acid*, and dilute with water to volume. These *Standard solutions* contain 1.0, 5.0, 10.0, and 15.0 µg/mL of calcium, respectively.

Sample solution: Transfer 250 mg of Magnesium Oxide, freshly ignited for 1 h in the temperature range of (800°–900°) ± 25°, to a beaker. Add 30 mL of *Dilute hydrochloric acid*, and stir until dissolved, heating if necessary. Transfer the solution so obtained to a 200-mL volumetric flask containing 4 mL of *Lanthanum solution*, and dilute with water to volume.

Blank solution: Transfer 4 mL of the *Lanthanum solution* and 10 mL of *Dilute hydrochloric acid* to a 200-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Nitrous oxide–acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank solution*

Using the calibration graph, determine the concentration, C , in µg/mL, of calcium in the *Sample solution*. Calculate the percentage of calcium in the portion of Magnesium Oxide taken:

$$\text{Result} = [(V/W) \times C \times F] \times 100$$

V = volume of the *Sample solution* (mL)

W = weight of Magnesium Oxide (mg)

C = concentration of calcium in the *Sample solution* (µg/mL)

F = conversion from µg/mL to mg/mL, 0.001

Acceptance criteria: NMT 1.1%

Delete the following:• **HEAVY METALS (231)**

Test preparation: Dissolve 2.0 g in 35 mL of 3 N hydrochloric acid, and evaporate the solution on a steam bath to dryness. Toward the end of the evaporation, stir frequently to disintegrate the residue so that finally a dry powder is obtained. Dissolve the residue in 20 mL of water, and evaporate to dryness in the same manner as before. Redissolve the residue in 20 mL of water, filter if necessary, and dilute with water to 40 mL. To 20 mL, add water to make 25 mL.

Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

• **IRON (241)**

Test preparation: Boil 40 mg with 5 mL of 2 N nitric acid for 1 min. Cool, dilute with water to 50 mL, and mix. Dilute 25 mL of this solution with water to 45 mL, and add 2 mL of hydrochloric acid.

Acceptance criteria: NMT 0.05%

SPECIFIC TESTS• **LOSS ON IGNITION (733)**

Sample: 500–1000 mg

Analysis: Transfer the *Sample* to a tared platinum crucible, and ignite in the temperature range of (800°–900°) ± 25° to constant weight.

Acceptance criteria: NMT 10.0%

• **BULK DENSITY AND TAPPED DENSITY OF POWDERS, Bulk Density, Method I (616):** Using the procedure specified in the chapter, determine the bulk density of Magnesium Oxide.**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label it to indicate its bulk density. The indicated density may be in the form of a range.

Magnesium Oxide Capsules**DEFINITION**

Magnesium Oxide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of magnesium oxide (MgO).

IDENTIFICATION• **A. IDENTIFICATION TESTS—GENERAL, Magnesium (191)**

Sample solution: Transfer the contents of 1 Capsule to a beaker. Add 10 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 min, and filter. Use the filtrate.

Acceptance criteria: Meet the requirements

ASSAY

• PROCEDURE

Eriochrome black indicator solution: Dissolve 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol.

Sample solution: Mix the contents of NLT 20 Capsules. Transfer a portion of the powder, equivalent to 500 mg of magnesium oxide, to a beaker. Add 20 mL of water, and slowly add 40 mL of 3 N hydrochloric acid with mixing. Heat the mixture to boiling, cool, and filter into a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the filter. Add water to volume.

Analysis: Transfer 20.0 mL of the *Sample solution* to a 400-mL beaker. Add 180 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of *Eriochrome black indicator solution*. Cool the solution to 3°–4° by immersion of the beaker in an ice bath. Remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 20 mL of water for the *Sample solution*, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.05 M edetate disodium consumed is equivalent to 2.015 mg of MgO.

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Analysis: Using atomic absorption spectrophotometry at a wavelength of 285.2 nm, determine the amount of MgO dissolved, using filtered portions of the solution under test, suitably diluted with *Medium* if necessary, in comparison with a standard solution having a known concentration of magnesium in the same *Medium*.

Tolerances: NLT 75% (Q) of the labeled amount of MgO is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

SPECIFIC TESTS

• ACID-NEUTRALIZING CAPACITY (301):

NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT 85.0% of the expected mEq value calculated from the results of the Assay is obtained. Each mg of MgO has an expected acid-neutralizing capacity value of 0.0492 mEq.

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in well-closed containers.

Magnesium Oxide Tablets

DEFINITION

Magnesium Oxide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of magnesium oxide (MgO).

IDENTIFICATION

• A. IDENTIFICATION TESTS—GENERAL, Magnesium (191)

Sample solution: Finely powder 1 Tablet. Transfer the powder to a beaker, add 10 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 min, and filter. Use the filtrate.

Acceptance criteria: Meet the requirements

ASSAY

• PROCEDURE

Eriochrome black indicator solution: Dissolve 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol.

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 500 mg of magnesium oxide, to a beaker. Add 20 mL of water, and slowly add 40 mL of 3 N hydrochloric acid, with mixing. Heat the mixture to boiling, cool, and filter into a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the filter. Add water to volume.

Analysis: Transfer 20.0 mL of the *Sample solution* to a 400-mL beaker. Add 180 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of *Eriochrome black indicator solution*. Cool the solution to 3°–4° by immersion of the beaker in an ice bath. Remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 20 mL of water for the *Sample solution*, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.05 M edetate disodium consumed is equivalent to 2.015 mg of MgO.

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 75 rpm

Time: 45 min

Analysis: Using atomic absorption spectrophotometry at a wavelength of 285.2 nm, determine the amount of MgO dissolved, using filtered portions of the solution under test, suitably diluted with *Medium* if necessary, in comparison with a standard solution having a known concentration of magnesium in the same *Medium*.

Tolerances: NLT 75% (Q) of the labeled amount of MgO is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

SPECIFIC TESTS

• ACID-NEUTRALIZING CAPACITY (301)

(where Tablets are labeled as intended for antacid use): NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT 85.0% of the expected mEq value calculated from the results of the Assay is obtained. Each mg of MgO has an expected acid-neutralizing capacity value of 0.0492 mEq.

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in well-closed containers.

Magnesium Phosphate

$\text{Mg}_3(\text{PO}_4)_2 \cdot 5\text{H}_2\text{O}$ 352.93
Phosphoric acid, magnesium salt (2:3), pentahydrate;
Magnesium phosphate (3:2) pentahydrate [10233-87-1].
 $\text{Mg}_3(\text{PO}_4)_2$ 262.86
[7757-87-1].

DEFINITION

Magnesium Phosphate, ignited at 425° to constant weight, contains NLT 98.0% and NMT 101.5% of $\text{Mg}_3(\text{PO}_4)_2$.

IDENTIFICATION

- **A.**
Sample: 200 mg
Analysis: Dissolve the *Sample* in 10 mL of 2 N nitric acid, and add, dropwise, ammonium molybdate TS.
Acceptance criteria: A greenish-yellow precipitate of ammonium phosphomolybdate is formed, and it is soluble in 6 N ammonium hydroxide.
- **B. IDENTIFICATION TESTS—GENERAL, Magnesium (191)**
Sample solution: Dissolve 0.1 g in 0.7 mL of 1 N acetic acid and 20 mL of water. Add 1 mL of ferric chloride TS, allow to stand for 5 min, and filter. Use 5 mL of the filtrate.
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
Sample: 200 mg, previously ignited at 425° to constant weight
Analysis: Dissolve the *Sample* in a mixture of 25 mL of water and 10 mL of 2 N nitric acid. Filter, if necessary. Wash any precipitate, add sufficient 6 N ammonium hydroxide to the filtrate to produce a slight precipitate, and then dissolve the precipitate by the addition of 1 mL of 2 N nitric acid. Adjust the temperature to 50°, add 75 mL of ammonium molybdate TS, and maintain the temperature at 50° for 30 min, stirring occasionally. Wash the precipitate once or twice with water by decantation, using 30–40 mL each time and passing the washings through a filter. Transfer the precipitate to the filter, and wash with potassium nitrate solution (1 in 100) until the last washing is not acid to litmus. Transfer the precipitate and filter to the precipitation vessel. Add 50 mL of water and 40.0 mL of 1 N sodium hydroxide VS, and agitate until the precipitate is dissolved. Add phenolphthalein TS, and then titrate the excess alkali with 1 N sulfuric acid VS. Each mL of 1 N sodium hydroxide is equivalent to 5.716 mg of $Mg_3(PO_4)_2$.
Acceptance criteria: 98.0%–101.5% on the previously ignited basis

IMPURITIES

- **ACID-INSOLUBLE SUBSTANCES**
[NOTE—Perform if an insoluble residue remains in the test for *Carbonate*.]
Analysis: Filter the solution, wash well with hot water until the last washing is free from chloride, and ignite the residue.
Acceptance criteria: The weight of the residue does not exceed 4 mg (NMT 0.2%).
- **SOLUBLE SUBSTANCES**
Sample: 2.0 g
Analysis: Digest the *Sample* with 100 mL of water on a steam bath for 30 min. Cool, add sufficient water to restore the original volume, mix, and filter. Evaporate 50 mL of the filtrate to dryness, and ignite gently to constant weight.
Acceptance criteria: The weight of the residue does not exceed 15 mg (NMT 1.5%).
- **CARBONATE**
Sample: 2.0 g
Analysis: Mix the *Sample* with 20 mL of water, and add hydrochloric acid, dropwise, to dissolve.
Acceptance criteria: No effervescence occurs when the acid is added.
- **CHLORIDE AND SULFATE, Chloride (221)**
Sample: 0.50 g
Analysis: Dissolve the *Sample* in 50 mL of 2 N nitric acid, and add 1 mL of silver nitrate TS.
Acceptance criteria: The turbidity does not exceed that produced by 1.0 mL of 0.020 N hydrochloric acid (NMT 0.14%).

- **CHLORIDE AND SULFATE, Sulfate (221)**
Sample: 0.50 g
Analysis: Dissolve the *Sample* in the smallest possible amount of 3 N hydrochloric acid, dilute with water to 48 mL, and add 2 mL of barium chloride TS.
Acceptance criteria: The turbidity does not exceed that produced by 3.0 mL of 0.020 N sulfuric acid (NMT 0.6%).
- **LIMIT OF NITRATE**
Sample: 0.20 g
Analysis: Mix the *Sample* with 5 mL of water, and add just sufficient hydrochloric acid to dissolve. Dilute with water to 10 mL, add 0.1 mL of indigo carmine TS, then add, with stirring, 10 mL of sulfuric acid.
Acceptance criteria: The blue color persists for NLT 5 min.
- **ARSENIC, Method I (211)**
Test preparation: Prepare as directed in the chapter, using 1.0 g and dissolving it first in just a sufficient amount of 3 N hydrochloric acid (about 9 mL).
Acceptance criteria: NMT 3 ppm
- **BARIUM**
Sample: 2.0 g
Analysis: Mix the *Sample* with 40 mL of water. Heat, add hydrochloric acid, dropwise, to dissolve, and then add 1 mL of acid in excess. Cool, dilute with water to 50 mL, and filter. To 5 mL of the filtrate add 1 mL of potassium sulfate TS.
Acceptance criteria: No turbidity is produced within 15 min.
- **CALCIUM**
Sample: 0.50 g
Analysis: Mix the *Sample* with 15 mL of water. Heat, and add sufficient hydrochloric acid, in small portions, to dissolve. Cool, add 6 N ammonium hydroxide, in small portions, to produce a slight permanent precipitate, then add 2 mL of 6 N acetic acid. Dilute with water to 25 mL, and filter. To 10 mL of the filtrate add 2 mL of ammonium oxalate TS.
Acceptance criteria: NMT a slight turbidity is produced within 5 min.
- **DIBASIC SALT AND MAGNESIUM OXIDE**
Sample: Ignite about 2.5 g to constant weight and use 2 g of the ignited salt.
Analysis: Dissolve the *Sample* by warming with 50.0 mL of 1 N hydrochloric acid VS. Cool, add 1 or 2 drops of methyl orange TS, and slowly titrate the excess 1 N hydrochloric acid VS with 1 N sodium hydroxide VS to a yellow color, vigorously shaking the mixture during the titration.
Acceptance criteria: 14.8–15.4 mL of 1 N hydrochloric acid is consumed for each g of the ignited salt.
- **LEAD (251)**
Test preparation: Dissolve 1.0 g in 20 mL of 3 N hydrochloric acid, evaporate on a steam bath to 10 mL, dilute with water to 20 mL, and cool.
Analysis: Proceed as directed in the chapter, using 5 mL of *Diluted Standard Lead Solution* (5 µg of Pb).
Acceptance criteria: NMT 5 ppm

Delete the following:

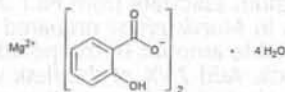
- **HEAVY METALS, Method I (231)**
Test preparation: Dissolve 0.67 g in 4.5 mL of 3 N hydrochloric acid, and dilute with water to 25 mL.
Acceptance criteria: NMT 30 ppm • (Official 1-Jan-2018)

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the test for the absence of *Escherichia coli*.
- **LOSS ON IGNITION (733):** Ignite a sample at 425° to constant weight; it loses 20.0%–27.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Magnesium Salicylate

$C_{14}H_{10}MgO_6 \cdot 4H_2O$ 370.59

$C_{14}H_{10}MgO_6$ 298.54

Magnesium, bis(2-hydroxybenzoato- O^1, O^2)-, tetrahydrate;
Magnesium salicylate (1:2), tetrahydrate [18917-95-8].
Anhydrous [18917-89-0].

DEFINITION

Magnesium Salicylate contains NLT 98.0% and NMT 103.0% of magnesium salicylate ($C_{14}H_{10}MgO_6 \cdot 4H_2O$).

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Magnesium (191):** Meets the requirements

ASSAY**• PROCEDURE**

Mobile phase: Methanol, phosphoric acid, and water (40: 0.1: 60), prepared by adding 1 mL of phosphoric acid to a solution containing 400 mL of methanol and 600 mL of water

Standard solution: 0.5 mg/mL of USP Magnesium Salicylate RS, in *Mobile phase*

Sample solution: 0.5 mg/mL of Magnesium Salicylate, in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 236 nm

Column: 4.6-mm \times 10-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.10%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of magnesium salicylate ($C_{14}H_{10}MgO_6 \cdot 4H_2O$) in the portion of Magnesium Salicylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Magnesium Salicylate RS in the *Standard solution* (mg/mL)

C_U = concentration of Magnesium Salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–103.0%

IMPURITIES**Delete the following:**

- **HEAVY METALS, Method I (231):** 40 ppm (Official 1-Jan-2018)

• ORGANIC IMPURITIES

Mobile phase: Methanol, phosphoric acid, and water (40: 0.1: 60), prepared by adding 1 mL of phosphoric acid to a solution containing 400 mL of methanol and 600 mL of water

Standard stock solution: 0.25 mg/mL of USP Magnesium Salicylate RS, 0.25 mg/mL of USP Salicylic Acid Related Compound A RS, 0.125 mg/mL of USP Salicylic Acid Related Compound B RS, and 0.05 mg/mL of USP Phenol RS, in *Mobile phase*

Standard solution: 0.005 mg/mL of USP Magnesium Salicylate RS, 0.005 mg/mL of USP Salicylic Acid Related Compound A RS, 0.0025 mg/mL of USP Salicylic Acid Related Compound B RS, and 0.001 mg/mL of USP Phenol RS, in *Mobile phase* prepared from *Standard stock solution*

Sample solution: 5 mg/mL of Magnesium Salicylate, in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 212 nm

Column: 4.6-mm \times 10-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between any two peaks

Relative standard deviation: NMT 3% for each peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of salicylic acid related compound A, salicylic acid related compound B, and phenol in the portion of Magnesium Salicylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of salicylic acid related compound A, salicylic acid related compound B, or phenol from the *Sample solution*

r_S = peak response of salicylic acid related compound A, salicylic acid related compound B, or phenol from the *Standard solution*

C_S = concentration of USP Salicylic Acid Related Compound A RS, USP Salicylic Acid Related Compound B RS, or USP Phenol RS in the *Standard solution* (mg/mL)

C_U = concentration of Magnesium Salicylate in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Magnesium Salicylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*

r_S = peak response of salicylic acid related compound B from the *Standard solution*

C_S = concentration of USP Salicylic Acid Related Compound B RS in the *Standard solution* (mg/mL)

C_U = concentration of Magnesium Salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid related compound A	0.3	0.1
Phenol	0.4	0.02
Salicylic acid related compound B	0.6	0.05
Salicylic acid	1.0	—
Any other individual impurity	—	0.05
Total impurities	—	0.2

SPECIFIC TESTS

MAGNESIUM CONTENT

Sample solution: Transfer 800 mg of Magnesium Salicylate to a 200-mL volumetric flask. Dissolve in and dilute with water to volume. Stir the solution continuously for 15 min, and filter, discarding the first 10 mL of the filtrate, into a flask.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Transfer 50.0 mL of the *Sample solution* to a 250-mL conical flask. Add 50 mL of water, 5 mL of ammonia-ammonium chloride buffer TS, and 0.15 mL of eriochrome black TS. Titrate with *Titrant* to a blue endpoint. Each mL of *Titrant* is equivalent to 1.215 mg of magnesium.

Acceptance criteria: 6.3%–6.7% of magnesium

WATER DETERMINATION, Method I (921): 17.5%–21.0%

ADDITIONAL REQUIREMENTS

PACKAGING AND STORAGE: Store in tight containers at controlled room temperature.

USP REFERENCE STANDARDS (11)

USP Magnesium Salicylate RS

USP Phenol RS

USP Salicylic Acid Related Compound A RS

4-Hydroxybenzoic acid.

$C_7H_6O_3$ 138.12

USP Salicylic Acid Related Compound B RS

4-Hydroxyisophthalic acid.

$C_8H_6O_5$ 182.13

Magnesium Salicylate Tablets

DEFINITION

Magnesium Salicylate Tablets contain an amount of magnesium salicylate ($C_{14}H_{10}MgO_6 \cdot 4H_2O$) equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of anhydrous magnesium salicylate ($C_{14}H_{10}MgO_6$).

IDENTIFICATION

A. The IR absorption spectrum of a potassium bromide dispersion of a quantity of finely powdered Tablets exhibits maxima at the same wavelengths as those of a similar preparation of USP Magnesium Salicylate RS.

B. IDENTIFICATION TESTS—GENERAL, Magnesium (191)

Sample solution: Prepare a 50-mg/mL magnesium salicylate solution from Tablets, and filter.

Acceptance criteria: Meet the requirements

C. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Mobile phase: Methanol, trifluoroacetic acid, and water (40:0.1:60), prepared by adding 1 mL of trifluoroacetic acid to a solution containing 400 mL of methanol and 600 mL of water

Standard solution: 0.05 mg/mL of anhydrous USP

Magnesium Salicylate RS in *Mobile phase*

Sample stock solution: Nominally 0.5 mg/mL of anhydrous magnesium salicylate from NLT 20 finely powdered Tablets in *Mobile phase* prepared as follows.

Transfer a suitable amount of the powder to a suitable volumetric flask. Add 75% of the flask volume of *Mobile phase*, and sonicate for 10 min. Allow the solution to cool to room temperature and then dilute with *Mobile phase* to volume.

Sample solution: Nominally 0.05 mg/mL of anhydrous magnesium salicylate in *Mobile phase*, from the *Sample stock solution*. Pass through a suitable filter of 0.20- μ m pore size, discarding the first 2–3 mL of the filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 212 nm

Column: 2.1-mm \times 5-cm; 1.7- μ m packing L1

Column temperature: 30°

Flow rate: 0.2 mL/min

Injection volume: 2 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.8

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anhydrous magnesium salicylate ($C_{14}H_{10}MgO_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of anhydrous USP Magnesium Salicylate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of anhydrous magnesium salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 120 min

Standard solution: USP Salicylic Acid RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium* if necessary.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum wavelength at about 296 nm

Blank: Water

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anhydrous magnesium salicylate ($C_{14}H_{10}MgO_6$) dissolved:

$$\text{Result} = (A_U/A_S) \times (1/L) \times (M_1/M_2) \times C_S \times V \times 100$$

A_U = absorbance from the *Sample solution*

A_S = absorbance from the *Standard solution*

L = nominal concentration of anhydrous magnesium salicylate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of anhydrous magnesium salicylate, 298.54

M_{r2} = twice the molecular weight of salicylic acid, 276.24

C_s = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of anhydrous magnesium salicylate ($C_{14}H_{10}MgO_6$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase: Methanol, trifluoroacetic acid, and water (40: 0.1: 60), prepared by adding 1 mL of trifluoroacetic acid to a solution containing 400 mL of methanol and 600 mL of water

System suitability solution: 0.5 mg/mL of USP Magnesium Salicylate RS, 0.5 µg/mL of USP Salicylic Acid Related Compound A RS, 0.5 µg/mL of USP Salicylic Acid Related Compound B RS, and 0.5 µg/mL of USP Phenol RS, in *Mobile phase*

Standard solution: 2.5 µg/mL of anhydrous USP Magnesium Salicylate RS in *Mobile phase*

Sample solution: Nominally 2.5 mg/mL of anhydrous magnesium salicylate from NLT 20 finely powdered Tablets in *Mobile phase* prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 80% of the flask volume of *Mobile phase*, and sonicate for 15 min. Allow the solution to cool to room temperature and then dilute with *Mobile phase* to volume. Centrifuge the solution, and use the supernatant.

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 212 nm

Column: 2.1-mm × 5-cm; 1.7-µm packing L1

Column temperature: 30°

Flow rate: 0.2 mL/min

Injection volume: 2 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between any two peaks, *System suitability solution*

Relative standard deviation: NMT 3%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of any individual unspecified impurity from the *Sample solution*

r_s = peak response of magnesium salicylate from the *Standard solution*

C_s = concentration of anhydrous USP Magnesium Salicylate RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of anhydrous magnesium salicylate in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid related compound A	0.3	— ^a
Phenol	0.4	— ^a
Salicylic acid related compound B	0.6	— ^a
Salicylic acid	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

^aThese are process impurities, which are included in the table for identification only. These impurities are controlled in the drug substance. They are not to be reported for the drug product and should not be included in the total impurities.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

• USP REFERENCE STANDARDS (11)

USP Magnesium Salicylate RS

USP Phenol RS

USP Salicylic Acid RS

USP Salicylic Acid Related Compound A RS

4-Hydroxybenzoic acid.

$C_7H_6O_3$ 138.12

USP Salicylic Acid Related Compound B RS

4-Hydroxyisophthalic acid.

$C_8H_6O_5$ 182.13

Magnesium Sulfate

$MgSO_4 \cdot xH_2O$

Sulfuric acid magnesium salt (1:1), hydrate;

Magnesium sulfate (1:1) monohydrate 138.36
[14168-73-1].

Magnesium sulfate (1:1) heptahydrate 246.47
[10034-99-8].

Anhydrous 120.37
[7487-88-9].

DEFINITION

Magnesium Sulfate, rendered anhydrous by ignition, contains NLT 99.0% and NMT 100.5% of $MgSO_4$.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Magnesium (191)* and *Sulfate (191)*

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Sample: 250 mg of the ignited Magnesium Sulfate obtained in the test for *Loss on Ignition*

Titrimetric system

Mode: Direct titration

Titrant: 0.05M edetate sodium VS

Endpoint detection: Colorimetric

Analysis: Dissolve the *Sample* in 100 mL of water and the minimum amount of 3 N hydrochloric acid required for a clear solution. Adjust the reaction of the solution (using pH indicator paper; see *Reagents, Indicators, and Solutions—Reagents—Indicator and Test Papers*) with 1 N sodium hydroxide to a pH of 7, add 5 mL of ammonia-ammonium chloride buffer TS and 0.15 mL of eriochrome black TS, and titrate with the *Titrant* to a

blue endpoint. Calculate the percentage of MgSO_4 in the portion of the ignited Magnesium Sulfate taken:

$$\text{Result} = [V \times N \times F \times 100] / W$$

- V = Sample titrant volume (mL)
 N = titrant molarity (mmol/mL)
 F = equivalency factor, 120.36 mg/mmol
 W = weight of Sample (mg)

Acceptance criteria: 99.0%–100.5% on the anhydrous by ignition basis

IMPURITIES

• LIMIT OF CHLORIDE (221)

Sample: 1.0 g

Acceptance criteria: The Sample shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.014%).

• LIMIT OF IRON (241)

Magnesium Sulfate intended for use in preparing nonparenteral dosage forms

Sample solution: Dissolve 0.50 g in 40 mL of water.

Analysis: Proceed as directed in the test for Iron (241).

Acceptance criteria: NMT 20 µg/g

Magnesium Sulfate intended for use in preparing parenteral dosage forms

[NOTE—Rinse all glassware used in this test with Dilute hydrochloric acid.]

Dilute hydrochloric acid: 1 mL of hydrochloric acid diluted with water to 1000 mL

Solution A: 500 mg/mL of ammonium acetate in water

Solution B: 13.4 mg/mL of ascorbic acid in water.

[NOTE—Use this solution on the day prepared.]

Color reagent: 3.8 mg/mL of 3-(2-pyridyl)-5,6-di-(2-furyl)-1,2,4-triazine-5',5''-disulfonic acid, disodium salt in Solution A. Shake by mechanical means if necessary. Use this solution on the day prepared.

Standard stock solution: 1.0 µg/mL of iron, from Standard Iron Solution in Dilute hydrochloric acid

Standard solutions: To three separate 50-mL volumetric flasks transfer 2.0, 5.0, and 10.0 mL of Standard stock solution, and dilute each with Dilute hydrochloric acid to 35 mL. These solutions contain 2.0, 5.0, and 10.0 µg of iron, respectively.

Sample solution: Transfer 10.0 g of Magnesium Sulfate to a 50-mL volumetric flask, add Dilute hydrochloric acid to 35 mL, and sonicate, if necessary, to dissolve.

Blank: Transfer 35 mL of Dilute hydrochloric acid to a 50-mL volumetric flask.

Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

Mode: UV-Vis

Analytical wavelength: 594 nm

Analysis

Samples: Standard solutions, Blank, and Sample solution

To each of the flasks containing the Standard solutions, the Sample solution, and the Blank add 5 mL of Solution B and 5 mL of Color reagent. Dilute each solution with Dilute hydrochloric acid to volume, mix, and allow to stand for 10 min.

Plot the absorbance values of the Standard solutions versus their iron contents in µg and draw the straight line best fitting the three plotted points. From the graph, determine the iron content, C , in µg, of the Sample solution.

Calculate the content, in µg/g, of iron in the portion of Magnesium Sulfate taken:

$$\text{Result} = C / W$$

C = content of iron in the Sample solution in µg, determined from the graph

W = weight of Magnesium Sulfate in the Sample solution (g)

Acceptance criteria: NMT 0.5 µg/g

• SELENIUM (291)

Test solution: 200 mg in 50 mL of 0.25 N nitric acid

Acceptance criteria: NMT 30 µg/g

Delete the following:

• HEAVY METALS (231)

Sample solution: 2 g in 25 mL of water

Acceptance criteria: NMT 10 ppm • (Official 1-Jan-2018)

SPECIFIC TESTS

• PH (791)

Sample solution: 50 mg/mL

Acceptance criteria: 5.0–9.2

• LOSS ON DRYING (731):

Dry a sample at 105° for 2 h; the anhydrous form loses NMT 2% of its weight.

• LOSS ON IGNITION (733)

Sample: 1 g

Analysis: Weigh the Sample in a crucible, heat at 105° for 2 h, then ignite in a muffle furnace at 450 ± 25° to constant weight.

Acceptance criteria

Monohydrate: Loses 13.0%–16.0% of its weight

Dried form: Loses 22.0%–28.0% of its weight

Heptahydrate: Loses 40.0%–52.0% of its weight

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in well-closed containers.

• LABELING:

The label states whether it is the monohydrate, the dried form, or the heptahydrate. Magnesium Sulfate intended for use in preparing parenteral dosage forms is so labeled. Magnesium Sulfate not intended for use in preparing parenteral dosage forms is so labeled. In addition, it may be labeled also as intended for use in preparing nonparenteral dosage forms.

Magnesium Sulfate Injection

DEFINITION

Magnesium Sulfate Injection is a sterile solution of Magnesium Sulfate in Water for Injection. It contains magnesium sulfate (MgSO_4) equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$).

IDENTIFICATION

• A. IDENTIFICATION TESTS—GENERAL, Magnesium (191) and Sulfate (191):

Meets the requirements

ASSAY

• PROCEDURE

Sample: A known volume of Injection equivalent to 250 mg of anhydrous magnesium sulfate

Titrimetric system

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis

Sample: Sample

Transfer the Sample to a beaker, and dilute with water to 100 mL. Adjust with 1 N sodium hydroxide to a pH of 7 (using pH indicator paper; see Reagents, Indicators, and Solutions—Indicators and Indicator Test Papers). Add 5 mL of ammonia–ammonium chloride buffer TS and 0.15 mL of eriochrome black TS. Titrate with Titrant to a blue endpoint. Each mL of Titrant consumed is equivalent to 12.32 mg of magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$).

Acceptance criteria: 93.0%–107.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.09 USP Endotoxin Unit/mg of magnesium sulfate.
- **PH (791)**
Sample solution: 50 mg/mL
Acceptance criteria: 5.5–7.0
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products (1)*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
- **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol/mL.
- **USP REFERENCE STANDARDS (11)**
USP Endotoxin RS

Magnesium Sulfate in Dextrose Injection

DEFINITION

Magnesium Sulfate in Dextrose Injection is a sterile solution of Magnesium Sulfate and Dextrose in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) and NLT 90.0% and NMT 110.0% of the labeled amount of dextrose ($\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$).

IDENTIFICATION

- **A.**
Sample solution 1: A suitable volume of Injection equivalent to 50 mg/mL of dextrose
Analysis 1: Add a few drops of the Sample solution to 5 mL of hot alkaline cupric tartrate TS.
Acceptance criteria 1: A copious red precipitate of cuprous oxide is formed.
Analysis 2: Proceed as directed in *Identification Tests—General (191)*, Magnesium.
Acceptance criteria 2: Meets the requirements

ASSAY

- **MAGNESIUM SULFATE**
Sample: A known volume of Injection equivalent to 250 mg of anhydrous magnesium sulfate
Titrimetric system
Mode: Direct titration
Titrant: 0.05 M edetate disodium VS
Endpoint detection: Visual
Analysis: Transfer the Sample to a beaker, and dilute with water to 100 mL. Adjust with 1 N sodium hydroxide to a pH of 7 using pH indicator paper (see *Reagents, Indicators, and Solutions—Indicator and Test Papers*), and add 5 mL of ammonia-ammonium chloride buffer TS and 0.15 mL of eriochrome black TS. Titrate with Titrant to a blue endpoint. Each mL of Titrant is equivalent to 12.32 mg of magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$).
Acceptance criteria: 93.0%–107.0% of the labeled amount of magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)
- **DEXTROSE**
Sample solution: Nominally 2 g/100 mL of dextrose prepared as follows. Transfer a suitable volume of Injection to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis: Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation (781)*). Calculate the percentage of the labeled amount of dextrose ($\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- a = observed angular rotation of the Sample solution ($^\circ$)
 l = length of the polarimeter tube, decimeter
 α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°
 C_U = nominal concentration of dextrose in the Sample solution, g/100 mL
 M_{r1} = molecular weight of dextrose monohydrate, 198.17
 M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–110.0% of the labeled amount of dextrose ($\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$)

IMPURITIES

- **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Sample solution: Nominally 2 mg/mL of dextrose ($\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$) in water from a suitable volume of Injection containing 1.0 g of dextrose in water

Instrumental conditions

Mode: UV
 Analytical wavelength: 284 nm
 Cell: 1 cm
 Blank: Water

Analysis

Samples: Sample solution and Blank
 Acceptance criteria: Absorbance of the Sample solution is NMT 0.25.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.039 USP Endotoxin Unit/mg of magnesium sulfate
- **PH (791):** 3.5–6.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products (1)*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **USP REFERENCE STANDARDS (11)**
USP Endotoxin RS

Magnesium Trisilicate

$2\text{MgO} \cdot 3\text{SiO}_2 \cdot x\text{H}_2\text{O}$ (anhydrous) 260.86
 Silicic acid ($\text{H}_4\text{Si}_3\text{O}_8$), magnesium salt (1:2), hydrate.
 Magnesium silicate hydrate ($\text{Mg}_2\text{Si}_3\text{O}_8 \cdot x\text{H}_2\text{O}$)
 [39365-87-2].
 Anhydrous [14987-04-3].

» Magnesium Trisilicate is a compound of Magnesium Oxide and silicon dioxide with varying proportions of water. It contains not less than 20.0 percent of magnesium oxide (MgO) and not less than 45.0 percent of silicon dioxide (SiO_2).

Packaging and storage—Preserve in well-closed containers.

Identification—

A: Mix about 500 mg with 10 mL of 3 N hydrochloric acid, filter, and neutralize the filtrate to litmus paper with

6 N ammonium hydroxide: the neutralized filtrate responds to the tests for *Magnesium* (191).

B: Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a Bunsen burner. Place the hot, transparent bead in contact with Magnesium Trisilicate, and again fuse: silica floats about in the bead, producing, upon cooling, an opaque bead with a web-like structure.

Water Determination, Method III (921)—Weigh accurately about 1 g in a tared platinum crucible provided with a cover. Gradually apply heat to the crucible at first, then strongly ignite to constant weight: it loses between 17.0% and 34.0% of its weight.

Soluble salts—Boil 10.0 g with 150 mL of water for 15 minutes. Cool to room temperature, allow the mixture to stand for 15 minutes, filter with the aid of suction, transfer the filtrate to a 200-mL volumetric flask, dilute with water to volume, and mix. Evaporate 50.0 mL of this solution, representing 2.5 g of the Trisilicate, in a tared platinum dish to dryness, and ignite gently to constant weight: the weight of the residue does not exceed 38.0 mg (1.5%).

Chloride (221)—A 20-mL portion of the diluted filtrate prepared in the test for *Soluble salts*, representing 1 g of Magnesium Trisilicate, shows no more chloride than corresponds to 0.75 mL of 0.020 N hydrochloric acid (0.055%).

Sulfate—Treat the residue obtained in the test for *Soluble salts* with 2 mL of hydrofluoric acid, and evaporate on a steam bath to dryness. Mix the residue with water, transfer to a filter, and wash, using approximately 50 mL of water for the complete procedure. Heat the filtrate to boiling, and add 0.1 mL of hydrochloric acid and 5 mL of barium chloride TS. Maintain the mixture near its boiling point for 1 hour, filter, wash the precipitate thoroughly with water, dry, and ignite to constant weight: the weight of the residue does not exceed 30 mg (0.5%).

Free alkali—Add 2 drops of phenolphthalein TS to 20 mL of the diluted filtrate prepared in the test for *Soluble salts*, representing 1 g of the Trisilicate: if a pink color is produced, not more than 1.0 mL of 0.10 N hydrochloric acid is required to discharge it.

Arsenic, Method I (211): 8 ppm.

Delete the following:

• **Heavy metals (231)**—Boil 2.67 g with a mixture of 50 mL of water and 5 mL of hydrochloric acid for 20 minutes, adding water to maintain the volume during the boiling. Add ammonium hydroxide until the mixture is only slightly acid to litmus paper. Filter with the aid of suction, and wash with 15 to 20 mL of water, combining the washing with the original filtrate. Add 2 drops of phenolphthalein TS, then add a slight excess of 6 N ammonium hydroxide. Discharge the pink color with dilute hydrochloric acid (1 in 100), then add 8 mL of dilute hydrochloric acid (1 in 100). Dilute with water to 100 mL, and use 25 mL of the solution for the test: the limit is 0.003%. (Official 1-Jan-2018)

Acid-consuming capacity—Weigh accurately about 200 mg into a glass-stoppered, 125-mL conical flask. Add 30.0 mL of 0.1 N hydrochloric acid VS and 20.0 mL of water. Place the flask in a bath maintained at 37°, and shake the mixture occasionally during a period of 4 hours but leave the mixture undisturbed during the last 15 minutes of the heating period. Cool to room temperature. To 25.0 mL of the supernatant add methyl red TS, and titrate the excess acid with 0.1 N sodium hydroxide VS. One g of Magnesium Trisilicate, calculated on the anhydrous basis, consumes not less than 140 mL and not more than 160 mL of 0.10 N hydrochloric acid.

Assay for magnesium oxide—Weigh accurately about 1.5 g, and transfer to a 250-mL conical flask. Add 50.0 mL of 1 N sulfuric acid VS, and digest on a steam bath for 1 hour. Cool to room temperature, add methyl orange TS,

and titrate the excess acid with 1 N sodium hydroxide VS. Each mL of 1 N sulfuric acid is equivalent to 20.15 mg of MgO.

Assay for silicon dioxide—Transfer about 700 mg of Magnesium Trisilicate, accurately weighed, to a small platinum dish. Add 10 mL of 1 N sulfuric acid, and heat on a steam bath to dryness, leaving the dish uncovered. Treat the residue with 25 mL of water, and digest on a steam bath for 15 minutes. Decant the supernatant through an ashless filter paper, with the aid of suction, and wash the residue, by decantation, three times with hot water, passing the washings through the filter paper. Finally transfer the residue to the filter, and wash thoroughly with hot water. Transfer the filter paper and its contents to the platinum dish previously used. Heat to dryness, incinerate, ignite strongly for 30 minutes, cool, and weigh. Moisten the residue with water, and add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 minutes, cool, and weigh: the loss in weight represents the weight of SiO₂.

Ratio of SiO₂ to MgO—Divide the percentage of SiO₂ obtained in the *Assay for silicon dioxide* by the percentage of MgO obtained in the *Assay for magnesium oxide*: the quotient obtained is between 2.10 and 2.37.

Magnesium Trisilicate Tablets

» Magnesium Trisilicate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of Mg₂Si₃O₈.

Packaging and storage—Preserve in well-closed containers.

Identification—

A: Powder 1 Tablet, add 10 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 minutes, and filter: the filtrate so obtained responds to the tests for *Magnesium* (191).

B: Wash the solids on the filter obtained in *Identification* test A with hot ammonium chloride solution (1 in 50), add 10 mL of 3 N hydrochloric acid, and filter. Transfer the filter paper and contents to a small platinum dish, ignite, cool in a desiccator, and weigh. Moisten the residue with water, and add 6 mL of hydrofluoric acid. Evaporate to dryness, ignite for 5 minutes, cool in a desiccator, and weigh: a loss of more than 10% in relation to the weight of the residue from the initial ignition indicates SiO₂.

Disintegration (701): 10 minutes, simulated gastric fluid TS being substituted for water in the test.

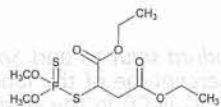
Uniformity of dosage units (905): meet the requirements.

Acid-neutralizing capacity (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

Assay—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 g of magnesium trisilicate, to a beaker, add 20 mL of water, and slowly add 40 mL of 3 N hydrochloric acid, with mixing. Heat the mixture to boiling, cool, and filter into a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the filter. Add water to volume, and mix. Transfer 20.0 mL of this solution to a 400-mL beaker, add 180 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia-ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution

to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 20 mL of water for the assay solution, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 6.521 mg of $\text{Mg}_2\text{Si}_3\text{O}_8$.

Malathion



$\text{C}_{10}\text{H}_{19}\text{O}_6\text{PS}_2$ 330.36
Butanedioic acid, [(dimethoxyphosphinothioyl)-thio]-, diethyl ester, (\pm)-;
Diethyl (\pm)-mercaptosuccinate, S-ester with O,O-dimethyl phosphorodithioate [121-75-5].

DEFINITION

Malathion contains NLT 98.0% and NMT 102.0% of malathion ($\text{C}_{10}\text{H}_{19}\text{O}_6\text{PS}_2$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197F)

ASSAY

• PROCEDURE

Mobile phase: Methanol and water (50:30)

Internal standard solution: 0.6 mg/mL of propylparaben in *Mobile phase*

Standard solution: 10 mg/mL of USP Malathion RS and 0.06 mg/mL of propylparaben from *Internal standard solution* in methanol

Sample solution: 10 mg/mL of Malathion and 0.06 mg/mL of propylparaben from *Internal standard solution* in methanol

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm \times 30-cm; 10- μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for propylparaben and malathion are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4 for propylparaben and malathion

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of malathion ($\text{C}_{10}\text{H}_{19}\text{O}_6\text{PS}_2$) in the portion of Malathion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of malathion to the internal standard from the *Sample solution*

R_S = peak response ratio of malathion to the internal standard from the *Standard solution*

C_S = concentration of USP Malathion RS in the *Standard solution* (mg/mL)

C_U = concentration of Malathion in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• LIMIT OF ISOMALATHION

Mobile phase: Methanol and water (50:30)

Standard solution: 0.1 mg/mL of USP Isomalathion RS in methanol

Sample solution: 20 mg/mL of Malathion in methanol

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm \times 30-cm; 10- μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for isomalathion and malathion are about 0.5 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of isomalathion in the portion of Malathion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of isomalathion from the *Sample solution*

r_S = peak response of isomalathion from the *Standard solution*

C_S = concentration of USP Isomalathion RS in the *Standard solution* (mg/mL)

C_U = concentration of Malathion in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.3%

SPECIFIC TESTS

• **SPECIFIC GRAVITY** (841): Between 1.220 and 1.240

• **WATER DETERMINATION, Method I** (921): NMT 0.1%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• USP REFERENCE STANDARDS (11)

USP Isomalathion RS

Butanedioic acid, [[methoxy(methylthio)phosphinyl]thio]-, diethyl ester.

$\text{C}_{10}\text{H}_{19}\text{O}_6\text{PS}_2$ 330.36

USP Malathion RS

Malathion Lotion

DEFINITION

Malathion Lotion is Malathion in a suitable isopropyl alcohol vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of malathion ($\text{C}_{10}\text{H}_{19}\text{O}_6\text{PS}_2$).

IDENTIFICATION

• **A.** The retention time of the major peak for malathion of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Solution A: Methyl ethyl ketone and *n*-hexane (4:1)

Internal standard solution: 2 mg/mL of parathion in *Solution A*

Standard stock solution: 2 mg/mL of USP Malathion RS in *Solution A*

Standard solution: 0.4 mg/mL of USP Malathion RS from the *Standard stock solution* and 0.4 mg/mL of parathion from the *Internal standard solution in Solution A*

Sample solution: Nominally 0.4 mg/mL of malathion and 0.4 mg/mL of parathion in *Solution A*, prepared as follows. Transfer a volume of Lotion, equivalent to 10 mg of malathion, to a 25-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with *Solution A* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m glass; packed with 5% G6 liquid phase on 110- to 120-mesh support S1A

Temperatures

Injector: 230°

Detector: 250°

Column: 190°

Carrier gas: Dry nitrogen

Flow rate: 15 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for malathion and parathion are 1.0 and about 1.3, respectively.]

Suitability requirements

Resolution: NLT 3.0 between malathion and parathion

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of malathion ($C_{10}H_{19}O_6PS_2$) in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of malathion to the internal standard from the *Sample solution*

R_S = peak response ratio of malathion to the internal standard from the *Standard solution*

C_S = concentration of USP Malathion RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of malathion in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• CONTENT OF ISOPROPYL ALCOHOL

Internal standard solution: Ethyl acetate and dehydrated alcohol (4:1)

Standard solution: Transfer 2.0 mL of isopropyl alcohol and 5.0 mL of *Internal standard solution* to a 200-mL volumetric flask, and dilute with ethyl acetate to volume.

Sample solution: Transfer a volume of Lotion, equivalent to 2.0 mL of isopropyl alcohol, to a 200-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with ethyl acetate to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m glass; packed with 110- to 120-mesh support S2

Temperatures

Injector: 200°

Detector: 220°

Column: 130°

Carrier gas: Dry nitrogen

Flow rate: 7 mL/min

Injection volume: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0% for the peak response ratio of isopropyl alcohol to the internal standard

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of isopropyl alcohol (C_3H_8O) in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of isopropyl alcohol to the internal standard from the *Sample solution*

R_S = peak response ratio of isopropyl alcohol to the internal standard from the *Standard solution*

C_S = concentration of isopropyl alcohol in the *Standard solution* (mg/mL)

C_U = nominal concentration of isopropyl alcohol in the *Sample solution* (mg/mL)

Acceptance criteria: 90%–110%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight glass containers.
- **LABELING:** The labeling states the percentage (v/v) of isopropyl alcohol in the Lotion.
- **USP REFERENCE STANDARDS (11)**
USP Malathion RS

Manganese Chloride

MnCl ₂ · 4H ₂ O	197.91
Manganese chloride (MnCl ₂) tetrahydrate; Manganese (2+) chloride tetrahydrate [13446-34-9].	
Anhydrous	125.84
[7773-01-5].	

DEFINITION

Manganese Chloride contains NLT 98.0% and NMT 101.0% of MnCl₂, calculated on the dried basis.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Yields white, curdy precipitate of silver chloride with silver nitrate TS, which is insoluble in nitric acid. After being washed with water, this precipitate is soluble in a slight excess of 6 N ammonium hydroxide.
- **B. IDENTIFICATION TESTS—GENERAL, Manganese (191):** Meets the requirements

ASSAY

• PROCEDURE

Sample: 425 mg

Analysis: Transfer the *Sample* to a 400-mL beaker, dissolve in 25 mL of water, add 300 mg of ammonium chloride and 0.5 g of hydroxylamine hydrochloride, and swirl to dissolve. Warm slightly on a hot plate, and dilute with water to 100 mL. Add 3 mL of triethanolamine and stir the solution, preferably using a magnetic stirrer. Begin the titration by adding 25 mL of 0.05 M edetate disodium VS, then add 10 mL of ammonia-ammonium chloride buffer TS, and 1 mL of eriochrome black TS. Continue to titrate with 0.05 M edetate disodium VS to

a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 6.292 mg of MnCl_2 .

Acceptance criteria: 98.0%–101.0% of MnCl_2 on the dried basis

IMPURITIES

• CHLORIDE AND SULFATE, Sulfate (221)

Sample: 2.0 g

Acceptance criteria: Shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.005%).

• IRON (241)

Sample solution: 2.0 g in 40 mL of water

Acceptance criteria: NMT 5 ppm

• ZINC

Sample solution: Dissolve 1 g in a mixture of 48 mL of water and 2 mL of sulfuric acid.

Analysis: To the *Sample solution*, add, slowly and with constant agitation, 1 mL of potassium ferrocyanide solution (1 in 100).

Acceptance criteria: No turbidity is produced within 5 min.

Delete the following:

• HEAVY METALS, Method I (231)

Sample solution: Dissolve 6.0 g in 30 mL of water.

[NOTE—Use 25 mL of this solution in the *Test Preparation* and use the remaining 5.0 mL in preparing the *Standard Preparation*.]

Acceptance criteria: NMT 5 ppm • (Official 1-Jan-2018)

SPECIFIC TESTS

• INSOLUBLE MATTER

Sample solution: Transfer 10 g to a 250-mL beaker, add 150 mL of water, cover the beaker, and heat to boiling.

Analysis: Digest the hot *Sample solution* on a steam bath for 1 h, and pass through a tared filtering crucible of fine pore size. Rinse the beaker with hot water, passing the rinsings through the filter, and finally wash the filter with additional hot water. Dry the filter at 105°.

Acceptance criteria: The residue weighs NMT 0.5 mg (0.005%).

• SUBSTANCES NOT PRECIPITATED BY AMMONIUM SULFIDE

Sample solution: Dissolve 2.0 g in 90 mL of water, add 5 mL of ammonium hydroxide, and warm the solution to 80°. Pass a stream of hydrogen sulfide through the solution for 30 min. Dilute with water to 100 mL, mix, and allow the precipitate to settle. Decant the supernatant through a filter of fine pore size, and transfer 50.0 mL to an evaporating dish that previously has been ignited and tared.

Analysis: Evaporate the filtrate to dryness, cool, add 0.5 mL of sulfuric acid, heat gently to remove the excess acid, and ignite at $800 \pm 25^\circ$ for 15 min.

Acceptance criteria: The weight of the residue is NMT 2.0 mg (NMT 0.2% as sulfate).

• PH (791)

Sample solution: 10 g in 200 mL of carbon dioxide- and ammonia-free water.

Acceptance criteria: 3.5–6.0

• LOSS ON DRYING (731):

Dry a sample at 50° for 2 h, then raise the temperature to 150° for 24 h: it loses 36.0%–38.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Manganese Chloride Injection

» Manganese Chloride Injection is a sterile solution of Manganese Chloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of manganese (Mn).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.

Labeling—Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.

USP Reference standards (11)—

USP Endotoxin RS

Identification—The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 279 nm when tested as directed for *Procedure* in the *Assay*.

Bacterial Endotoxins Test (85)—It contains not more than 0.45 USP Endotoxin Unit per μg of manganese.

pH (791): between 1.5 and 2.5.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products (1)*.

Assay—

Sodium chloride solution—Dissolve 1.8 g of sodium chloride in water, dilute with water to 2000 mL, and mix.

Manganese stock solution—Transfer 1.000 g of manganese to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, dilute with 0.1 N hydrochloric acid to volume, and mix. This solution contains 1000 μg of manganese per mL. Store in a polyethylene bottle.

Standard preparations—Pipet 10 mL of the *Manganese stock solution* into a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 4.0, 5.0, and 6.0 mL, respectively, of this solution to separate 50-mL volumetric flasks, containing 10 mL of *Sodium chloride solution*, dilute the contents of each flask with water to volume, and mix. These *Standard preparations* contain, respectively, 1.6, 2.0, and 2.4 μg of manganese per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 1 mg of manganese, to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of this solution into a 50-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the Injection, calculate the amount, in mg, of sodium chloride in the initial dilution, and add sufficient *Sodium chloride solution* to bring the total sodium chloride content in this flask to 9 mg. Dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the manganese emission line of 279 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy (852)*) equipped with a manganese hollow-cathode lamp and an air-acetylene flame, using a dilution of *Sodium chloride solution* (1:5) as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in μg per mL, of manganese, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, in μg per mL, of manganese in the

Assay preparation. Calculate the quantity, in mg, of manganese in each mL of the Injection taken by the formula:

$$0.5C/V$$

in which C is the concentration, in μg per mL, of manganese in the *Assay preparation*; and V is the volume, in mL, of Injection taken.

Manganese Chloride for Oral Solution

» Manganese Chloride for Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of manganese (Mn). It may contain one or more suitable flavors, sweetening agents, thickening agents, and stabilizers.

Packaging and storage—Preserve in tight, light-resistant, single-dose containers.

Labeling—The label contains directions for constitution of the powder and states the amount of manganese in a given volume of the Oral Solution obtained after constitution.

Identification—It meets the requirements of the tests for Chloride (191) and for Manganese (191).

Uniformity of dosage units (905)—

FOR POWDER PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698)—

FOR POWDER PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

pH (791): between 6.0 and 8.0, when constituted to 300 mL with water.

Osmolality and Osmolarity (785): 230 mOsmol pH 6.0 to 8.0.

Assay—

Sodium chloride solution, Manganese stock solution, and Standard preparations—Prepare as directed in the *Assay under Manganese Chloride Injection*.

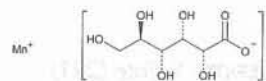
Assay preparation—Constitute the Manganese Chloride for Oral Solution as directed in the labeling. Transfer about 25 mL, accurately measured, of the constituted Manganese Chloride for Oral Solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Proceed as directed for *Assay preparation* in the *Assay under Manganese Chloride Injection*, beginning with "Pipet 10 mL of this solution."

Procedure—Proceed as directed in the *Assay under Manganese Chloride Injection*. Calculate the quantity, in μg , of manganese in each mL of the constituted for Oral Solution taken by the formula:

$$500C/V$$

in which C is the concentration, in μg per mL, of the manganese in the *Assay preparation*; and V is the volume, in mL, of the constituted Manganese Chloride for Oral Solution taken.

Manganese Gluconate



$\text{C}_{12}\text{H}_{22}\text{MnO}_{14}$ 445.23

$\text{C}_{12}\text{H}_{22}\text{MnO}_{14} \cdot 2\text{H}_2\text{O}$ 481.26

Bis(D-gluconato-O¹,O²) manganese;

Manganese D-gluconate (1:2).

Anhydrous [6485-39-8].

DEFINITION

Manganese Gluconate is dried or contains two molecules of water of hydration. It contains NLT 98.0% and NMT 102.0% of manganese gluconate ($\text{C}_{12}\text{H}_{22}\text{MnO}_{14}$), calculated on the anhydrous basis.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL, Manganese (191):** A 50-mg/mL solution meets the requirements.

• **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: 10 mg/mL of Manganese Gluconate, heating in a water bath at 60°, if necessary, to dissolve

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 μL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_f value to that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Sample: 700 mg of Manganese Gluconate

Blank: 50 mL of water

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 50 mL of water. Add 1 g of ascorbic acid and 10 mL of ammonia-ammonium chloride buffer TS and 0.1 mL of eriochrome black TS. Titrate with the *Titrant* until the solution is deep blue in color. Perform the blank determination.

Calculate the percentage of manganese gluconate ($\text{C}_{12}\text{H}_{22}\text{MnO}_{14}$) in the *Sample* taken:

$$\text{Result} = \{(V_S - V_B) \times M \times F/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

M = actual molarity of the *Titrant* (mmol/mL)

F = equivalency factor, 445.2 mg/mmol

W = *Sample* weight (mg)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **CHLORIDE AND SULFATE, Chloride (221)**
Standard: 0.70 mL of 0.020 N hydrochloric acid
Sample: 1.0 g of Manganese Gluconate
Acceptance criteria: NMT 0.05%
- **CHLORIDE AND SULFATE, Sulfate (221)**
Standard: 4.0 mL of 0.020 N sulfuric acid
Sample: 2.0 g of Manganese Gluconate
Acceptance criteria: NMT 0.2%

Delete the following:

- **HEAVY METALS (231)**
Test preparation: Dissolve 1 g of Manganese Gluconate in 10 mL of water, add 6 mL of 3 N hydrochloric acid, and dilute with water to 25 mL.
Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

LEAD

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

Ascorbic acid–sodium iodide solution: 100 mg/mL of ascorbic acid and 192.5 mg/mL of sodium iodide

Trioctylphosphine oxide solution: 50 mg/mL of trioctylphosphine oxide in 4-methyl-2-pentanone.

[CAUTION—This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.]

Standard solution: Transfer 5.0 mL of lead nitrate stock solution TS, to a 100-mL volumetric flask. Dilute with water to volume. Transfer 2.0 mL of the resulting solution to a 50-mL volumetric flask. Add 10 mL of 9 N hydrochloric acid and 10 mL of water. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Standard solution*, and it contains 2.0 µg/mL of lead.

Sample solution: To a 50-mL volumetric flask add 1.0 g of Manganese Gluconate, 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Sample solution*.

Blank: To a 50-mL volumetric flask add 10 mL of 9 N hydrochloric acid, 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution*. Shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic layer is the *Blank*, and it contains 0 µg/mL of lead.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 283.3 nm

Lamp: Lead hollow-cathode

Flame: Air–acetylene

System suitability

Samples: *Standard solution* and *Blank*

Suitability requirements: The absorbance of the *Standard solution* and the absorbance of the *Blank* are significantly different.

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*
Concomitantly determine the absorbances of the *Blank*, *Standard solution*, and the *Sample solution*. Use the *Blank* to set the instrument to zero.

Acceptance criteria: The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 10 ppm).

REDUCING SUBSTANCES

Sample: 1.0 g of Manganese Gluconate

Blank: Proceed as directed in the *Analysis*, omitting the *Sample*.

Titrimetric system

(See *Titrimetry* (541).)

Mode: Residual titration

Titrant: 0.1 N iodine VS

Back-titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 250-mL conical flask, dissolve in 10 mL of water, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid, and titrate with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the blank determination.

Calculate the percentage of reducing substances (as dextrose) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F] / W\} \times 100$$

V_B = Back-titrant volume consumed by the *Blank* (mL)

V_S = Back-titrant volume consumed by the *Sample* (mL)

N = Back-titrant normality (mEq/mL)

F = equivalency factor, 27 mg/mEq

W = Sample weight (mg)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

• WATER DETERMINATION, Method I (921)

Analysis: Proceed as directed in the chapter. Maintain the mixture containing the *Test preparation* at 50°, and stir for 30 min before titrating with the *Reagent*.

Acceptance criteria

Where labeled as the dried form: 3.0%–9.0%

Where labeled as the dihydrate: 6.0%–9.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** The label indicates whether it is the dried or the dihydrate form.
- **USP REFERENCE STANDARDS (11)**
USP Potassium Gluconate RS

Manganese Sulfate

MnSO₄ · H₂O 169.02
Sulfuric acid, manganese(2+) salt (1:1) monohydrate;
Manganese(2+) sulfate (1:1) monohydrate [10034-96-5].
Anhydrous 151.00
[7785-87-7].

DEFINITION

Manganese Sulfate contains NLT 98.0% and NMT 102.0% of MnSO₄ · H₂O.

IDENTIFICATION

• A. IDENTIFICATION TESTS—GENERAL, Manganese (191)

Sample solution: 100 mg/mL

Acceptance criteria: Meets the requirements

• B. IDENTIFICATION TESTS—GENERAL, Sulfate (191)

Sample solution: 100 mg/mL

Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Sample: 350 mg

Analysis: Dissolve the *Sample* in 200 mL of water. Add 10 mg of ascorbic acid. Begin the titration by adding 25 mL of 0.05 M edetate disodium VS using a suitable buret, then add 10 mL of ammonia–ammonium chloride buffer TS and 0.15 mL of eriochrome black TS.

Complete the titration with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 8.451 mg of MnSO₄ · H₂O.

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• SUBSTANCES NOT PRECIPITATED BY AMMONIUM SULFIDE

Sample: 2.0 g

Analysis: Dissolve the *Sample* in 90 mL of water, add 5 mL of ammonium hydroxide, warm the solution, and pass hydrogen sulfide through the solution for about 30 min. Dilute with water to 100 mL, mix, and allow the precipitate to settle. Decant the supernatant through a filter, transfer 50 mL of the clear filtrate to a tared dish, evaporate to dryness, and ignite, gently at first and finally at 800 ± 25°.

Acceptance criteria: The weight of the residue does not exceed 5 mg (NMT 0.5%).

SPECIFIC TESTS

• LOSS ON IGNITION (733)

Analysis: Ignite a sample at 450° to constant weight: it loses 10.0%–13.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

Manganese Sulfate Injection

» Manganese Sulfate Injection is a sterile solution of Manganese Sulfate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of manganese (Mn).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.

Labeling—Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.

USP Reference standards (11)—

USP Endotoxin RS

Identification—The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 279 nm when tested as directed for *Procedure* in the *Assay*.

Bacterial Endotoxins Test (85)—It contains not more than 0.45 USP Endotoxin Unit per µg of manganese.

pH (791): between 2.0 and 3.5.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

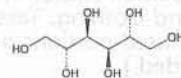
Sodium chloride solution, *Manganese stock solution*, and *Standard preparations*—Prepare as directed in the *Assay* under *Manganese Chloride Injection*.

Assay preparation—Transfer an accurately measured volume of *Injection*, equivalent to about 1 mg of manganese, to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of this solution into a 50-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Manganese Chloride Injection*.

Mannitol

Portions of the monograph text that are national USP text, and are not part of the harmonized text, are marked with symbols (✱) to specify this fact.



C₆H₁₄O₆

D-Mannitol [69-65-8].

182.17

DEFINITION

Mannitol contains NLT 97.0% and NMT 102.0% of mannitol (C₆H₁₄O₆), calculated on the dried basis.

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

If the spectra shows differences, proceed as directed.

Standard solution: Dissolve 25 mg of USP Mannitol RS in a glass vial with 0.25 mL of distilled water without heating. The solution is clear. Evaporate to dryness by one of the following methods. Heat in a microwave oven with a power range of 600–700 W for 20 min, or heat in an oven at 100° for 1 h, then gradually apply vacuum until a dry residue is obtained. Non-sticky, white, or slightly yellowish powders are obtained.

Sample solution: Dissolve 25 mg of Mannitol in a glass vial with 0.25 mL of distilled water without heating. The solution is clear. Evaporate to dryness by one of the following methods. Heat in a microwave oven with a power range of 600–700 W for 20 min, or heat in an oven at 100° for 1 h, then gradually apply vacuum until a dry residue is obtained. Non-sticky, white, or slightly yellowish powders are obtained.

Analysis: Record new spectra using the residues from the *Standard solution* and the *Sample solution*.

ASSAY

• PROCEDURE

Mobile phase: Degassed water

System suitability solution A: 25.0 mg/mL each of sorbitol and USP Mannitol RS

System suitability solution B: 1.0 mg/mL each of maltitol and isomalt

Standard solution A: 50.0 mg/mL of USP Mannitol RS

Standard solution B: Dilute 2.0 mL of the *Sample solution* with water to 100.0 mL.

Standard solution C: Dilute 0.5 mL of *Standard solution B* with water to 20.0 mL.

Sample solution: 50.0 mg/mL of Mannitol

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 7.8-mm × 30-cm; packing L19

Temperatures

Detector: 40° (maintain at a constant temperature)

Column: 85 ± 2°

Flow rate: 0.5 mL/min

Injection volume: 20 µL

Run time: NLT 1.5 times the retention time of the mannitol peak. [NOTE—The retention time for mannitol is about 20 min.]

System suitability

Samples: *System suitability solution A*, *System suitability solution B*, *Standard solution B*, and *Standard solution C*

Suitability requirements

Resolution: NLT 2.0 between sorbitol and mannitol, *System suitability solution A*

Analysis

Samples: *Standard solution A* and *Sample solution*

Calculate the percentage of mannitol (C₆H₁₄O₆) in the portion of Mannitol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from *Standard solution A*

C_S = concentration of USP Mannitol RS in *Standard solution A* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

• RELATED SUBSTANCES

Mobile phase, System suitability solution A, System suitability solution B, Standard solution B, Standard solution C, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution B*, *Standard solution C*, and *Sample solution*

Acceptance criteria: See *Table 1* for the relative retention times.

Table 1

Name	Relative Retention Time
Isomalt (1st peak)	0.60
Maltitol	0.69
Isomalt (2nd peak)	0.73
Mannitol	1.0
Sorbitol	1.2

[NOTE—Impurity A—Sorbitol; Impurity B—Maltitol; Impurity C—Isomalt.]

[NOTE—Isomalt elutes in two peaks.]

[NOTE—Coelution of impurity B and the second peak of impurity C may be observed.]

Disregard limit: NMT 0.05%; any peak NMT the area of the principal peak obtained with *Standard solution C*

Sorbitol: NMT 2.0%; NMT the area of the principal peak obtained with *Standard solution B*

Sum of isomalt and maltitol: NMT 2.0%; NMT the area of the principal peak obtained with *Standard solution B*

Unspecified impurities: NMT 0.10% for each impurity; NMT twice the area of the principal peak obtained with *Standard solution C*

Total impurities: 2.0%; NMT the area of the principal peak obtained with *Standard solution B*

• REDUCING SUGARS

Ferric sulfate solution: Dissolve 50 g of ferric sulfate in an excess of water, add 200 mL of sulfuric acid, and dilute with water to 1000 mL.

Copper sulfate solution: 69.2 mg/mL of copper sulfate (CuSO₄ · 5H₂O) in water

Sodium potassium tartrate solution: Dissolve 173 g of sodium potassium tartrate (C₄H₄KNaO₆ · 4H₂O) and 50 g of sodium hydroxide in 400 mL of water. Heat to boiling, allow to cool, and dilute with carbon dioxide-free water to 500 mL.

Cupri-tartaric solution: Mix equal volumes of *Copper sulfate solution* and *Sodium potassium tartrate solution* immediately before use.

Sample: 7.0 g

Analysis: To the *Sample* add 13 mL of water. Boil gently with 40 mL of *Cupri-tartaric solution* for 3 min, and allow to stand for about 2 min. A precipitate is formed. Pass through a sintered-glass filter (10–16 µm) coated with diatomaceous earth or a sintered-glass filter (5–10 µm). Wash the precipitate with hot water (at about 50°–60°) until the washing is no longer alkaline, and pass the washings through the filter described above. Discard all the filtrate at this step. Immediately dissolve the precipitate in 20 mL of *Ferric sulfate solution*, pass through the filter described above in a clean flask, and wash the filter with 15–20 mL of water. Combine the washings and the filtrate, heat to 80°, and titrate with 0.02 M potassium permanganate VS.

Acceptance criteria: NMT 0.1%, expressed as glucose; NMT 3.2 mL of 0.02 M potassium permanganate VS is required to change the color of the solution. The green color turns to pink, and the color persists at least 10 s.

• NICKEL

Sample solution: Suspend 10.0 g of Mannitol in 30 mL of dilute acetic acid [115–125 g/L of acetic acid (C₂H₄O₂)], add water, and shake to dissolve. Dilute with water to 100.0 mL. Add 2.0 mL of a saturated solution of ammonium pyrrolidinedithiocarbamate (C₅H₁₂N₂S₂) (about 10 g/L) and 10.0 mL of water-saturated methyl isobutyl ketone (C₆H₁₂O, 4-methyl-2-pentanone), and then shake for 30 s protected from bright light. Allow the layers to separate, and use the methyl isobutyl ketone layer.

Blank solution: Treat water-saturated methyl isobutyl ketone as described for preparation of the *Sample solution*, omitting the mannitol.

Standard solutions: Prepare three reference solutions in the same manner as the *Sample solution* but adding 0.5, 1.0, and 1.5 mL, respectively, of nickel standard solution TS [10 ppm nickel (Ni)] in addition to the 10.0 g of the substance to be examined.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 232.0 nm

Lamp: Nickel hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Sample solution* and *Standard solutions*

Set the zero of the instrument using the blank. Record the average of the steady readings for each of the *Standard solutions* and the *Sample solution*. Between each measurement rinse with water, and ascertain that the reading returns to zero with the blank. Plot the absorbances of the *Standard solutions* and the *Sample solution* versus the added quantity of nickel. Extrapolate

late the line joining the points on the graph until it meets the concentration axis. The distance between this point and the intersection of the axes represents the concentration of nickel in the *Sample solution*.

Acceptance criteria: NMT 1 µg/g

SPECIFIC TESTS

• MELTING RANGE OR TEMPERATURE (741), Class I

Melting point: 165°–170°

• APPEARANCE OF SOLUTION

Hydrazine sulfate solution: 10.0 mg/mL of hydrazine sulfate. Allow to stand for 4–6 h.

Methenamine solution: 2.5 g of methenamine in 25 mL of water, in a ground-glass-stoppered flask

Primary opalescent suspension: To the *Methenamine solution*, add 25.0 mL of the *Hydrazine sulfate solution*. Mix, and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Opalescence standard: Dilute 15.0 mL of the *Primary opalescent suspension* with water to 1000.0 mL. This suspension is freshly prepared and may be stored for up to 24 h.

Reference suspension: To 5.0 mL of *Opalescence standard* add 95.0 mL of water. Mix, and shake before use.

Standard solution: Pipet 3.0 mL of ferric chloride CS, 3.0 mL of cobaltous chloride CS, and 2.4 mL of cupric sulfate CS into a 1-L volumetric flask. Dilute with 1% (w/v) hydrochloric acid to volume.

Sample solution: 100.0 mg/mL of Mannitol

Analysis: Compare the color, clarity, and opalescence of equal volumes of the *Reference suspension*, *Standard solution*, and *Sample solution*.

Acceptance criteria: The *Sample solution* is clear and colorless; its clarity is the same as that of water, or its opalescence is not more pronounced than that of the *Reference suspension*, and it is not more intensely colored than the *Standard solution*.

• LOSS ON DRYING (731)

Sample: 1.000 g

Analysis: Dry the *Sample* at 105° for 4 h.

Acceptance criteria: NMT 0.5%

• CONDUCTIVITY

Sample: 20.0 g

Analysis: Dissolve the *Sample* in carbon dioxide-free water prepared from distilled water by heating to 40°–50°, and dilute with the same solvent to 100 mL. After cooling, measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Acceptance criteria: NMT 20 µS/cm at 25°

• MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):

The total aerobic microbial count (TAMC) is NMT 10³ cfu/g, and the total combined molds and yeasts count is NMT 10² cfu/g. It meets the requirements of the test for absence of *Escherichia coli*. If intended for use in the manufacture of parenteral dosage forms, the TAMC is NMT 10² cfu/g.

• BACTERIAL ENDOTOXINS TEST (85):

If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins, less than 4 IU/g for parenteral dosage forms with a concentration of 100 g/L or less of mannitol, and less than 2.5 IU/g for parenteral dosage forms with a concentration of more than 100 g/L of mannitol

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in well-closed containers.

• LABELING

The label states, where applicable, the maximum concentration of bacterial endotoxins.

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

• USP REFERENCE STANDARDS (11)

USP Endotoxin RS

USP Mannitol RS

Mannitol Injection

» Mannitol Injection is a sterile solution, which may be supersaturated, of Mannitol in Water for Injection. It may require warming or autoclaving before use if crystallization has occurred. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of mannitol (C₆H₁₄O₆). It contains no antimicrobial agents.

Packaging and storage—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL. The label also states that it should be warmed before use to dissolve any crystals that may have formed.

USP Reference standards (11)—

USP Endotoxin RS

USP Mannitol RS

Identification—

A: Evaporate a portion of Injection on a steam bath to dryness, and dry the residue at 105° for 4 hours. To 3 mL of freshly prepared solution of catechol in water (1 in 10) add 6 mL of sulfuric acid with cooling. Place 3 mL of this solution in each of two separate test tubes. To one tube add 0.3 mL of water (reagent blank) and to the other add 0.3 mL of a solution of it in water (1 in 10). Heat the tubes over an open flame for about 30 seconds: the solution in the tube containing mannitol is dark pink or wine red, and the solution in the tube containing the reagent blank is light pink.

B: The retention time for the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Specific rotation (781)—+137° to +145°. Transfer an accurately measured volume of Injection, equivalent to about 1 g of mannitol as determined by the *Assay*, to a 100-mL volumetric flask. Add 40 mL of a 1-in-10 ammonium molybdate solution, previously filtered if necessary. Add 20 mL of 1 N sulfuric acid, and dilute with water to volume.

Bacterial Endotoxins Test (85)—It contains not more than 0.04 USP Endotoxin Unit per mg of mannitol where the labeled amount of mannitol in the Injection is 10% or less, and not more than 2.5 USP Endotoxin Units per g of mannitol where the labeled amount of mannitol in the Injection is greater than 10%.

pH (791): between 4.5 and 7.0, determined potentiometrically, on a portion to which 0.30 mL of saturated potassium chloride solution has been added for each 100 mL, and which previously has been diluted with water, if necessary, to a concentration of not more than 5% of mannitol.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Mobile phase—Use degassed water.

Resolution solution—Dissolve sorbitol and USP Mannitol RS in water to obtain a solution having concentrations of about 4.8 mg per mL of each.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a refractive index detector that is maintained at a constant temperature and a 4-mm × 25-cm column that contains packing L19. The column temperature is maintained at a temperature between 30° and 85° controlled within ±2° of the selected temperature, and the flow rate is about 0.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%. In a similar manner, chromatograph the *Resolution solution*: the resolution, *R*, between the sorbitol and mannitol peaks is not less than 2.0.

Standard preparation—Dissolve an accurately weighed quantity of USP Mannitol RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 5 mg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 500 mg of mannitol, to a 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of mannitol (C₆H₁₄O₆) in each mL of the Injection taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which *V* is the volume, in mL, of Injection taken; and the other terms are as defined therein.

Mannitol in Sodium Chloride Injection

» Mannitol in Sodium Chloride Injection is a sterile solution of Mannitol and Sodium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of C₆H₁₄O₆ and NaCl. It contains no antimicrobial agents.

Labeling—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

USP Reference standards (11)—

USP Endotoxin RS

USP Mannitol RS

Identification—

A: Evaporate a portion of Injection on a steam bath to dryness, and dry the residue at 105° for 4 hours: the residue responds to the *Identification test A* under *Mannitol Injection*.

B: It responds to the tests for *Sodium* (191) and for *Chloride* (191).

Bacterial Endotoxins Test (85)—It contains not more than 0.04 USP Endotoxin Unit per mg of mannitol.

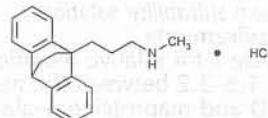
pH (791): between 4.5 and 7.0.

Other requirements—It meets the requirements for *Packaging and storage* under *Mannitol Injection*. It meets also the requirements under *Injections and Implanted Drug Products* (1).

Assay for mannitol—Proceed with Injection as directed in the *Assay* under *Mannitol Injection*.

Assay for sodium chloride—Proceed with Injection as directed in the *Assay* under *Sodium Chloride Injection*.

Maprotiline Hydrochloride



C₂₀H₂₃N · HCl 313.86
9,10-Ethanoanthracene-9(10*H*)-propanamine, *N*-methyl-, hydrochloride;
N-Methyl-9,10-ethanoanthracene-9(10*H*)-propylamine hydrochloride [10347-81-6].

DEFINITION

Maprotiline Hydrochloride contains NLT 99.0% and NMT 101.0% of the labeled amount of maprotiline hydrochloride (C₂₀H₂₃N · HCl), calculated on the dried basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

• **B. ULTRAVIOLET ABSORPTION** (197U)

Sample solution: 100 µg/mL in methanol

Acceptance criteria: Absorptivities at 266 nm and 272 nm, calculated on the dried basis, do not differ by more than 3.0%.

• **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191)

Sample solution: 5 mg/mL

Acceptance criteria: Responds to the tests when tested as specified for alkaloidal hydrochloride

ASSAY

• **PROCEDURE**

Sample solution: 600 mg of Maprotiline Hydrochloride in 25 mL of mercuric acetate TS

Blank: Mercuric acetate TS

Titrimetric system

(See *Titrimetry* (541).)

Mode: Potentiometric

Titrant: 0.1 N perchloric acid VS

Analysis: Titrate the *Sample solution* with *Titrant* using a glass electrode and a calomel electrode containing saturated lithium chloride in glacial acetic acid. Perform a blank determination. Each mL of 0.1 N perchloric acid is equivalent to 31.39 mg of maprotiline hydrochloride (C₂₀H₂₃N · HCl).

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

• **HEAVY METALS, Method II** (231): NMT 10 ppm • (Official 1-

Jan-2018)

• **ORGANIC IMPURITIES**

Mobile phase: Dissolve 0.6 g of ammonium acetate in 200 mL of water, and add 2 mL of a 70-g/L ammonia solution, 150 mL of 2-propanol, and 650 mL of methanol. The resulting apparent pH value is 8.2–8.4.

Standard solution: 2 µg/mL of USP Maprotiline Hydrochloride RS in *Mobile phase*

System suitability solution: 1 mg/mL of USP Maprotiline Hydrochloride RS and 0.1 mg/mL of USP Maprotiline Related Compound D RS in *Mobile phase*

Sample solution: 1 mg/mL of Maprotiline Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 272 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 1.5 times the retention time of maprotiline

System suitability

Sample: *System suitability solution*

Suitability requirements

[NOTE—See *Table 1* for relative retention times.]

Resolution: 1.8–3.2 between the maprotiline related compound D and maprotiline peaks

[NOTE—If necessary, adjust the pH of the *Mobile phase*, in steps of 0.1 pH unit, by adding a 50% v/v solution of acetic acid if the resolution is less than 1.8, or by adding a 70-g/L solution of ammonia if the resolution is greater than 3.2.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Maprotiline Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak area of any impurity from the *Sample solution*

r_s = peak area of maprotiline from the *Standard solution*

C_s = concentration of USP Maprotiline Hydrochloride RS in the *Standard solution* (mg/mL)

C_u = concentration of Maprotiline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any peak representing less than 0.05% of the area of the main peak.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Maprotiline acrylaldehyde analog ¹	0.3	0.2
Maprotiline dimer ²	0.5	0.2
Desmethymaprotiline ³	0.7	0.2
Maprotiline related compound D ⁴	0.8	0.2
Maprotiline	1.0	—
N-Methylmaprotiline ⁵	1.3	0.2

¹ 3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)acrylaldehyde (EP Impurity A).

² N-Methyl-N,N-bis[3-(9,10-dihydro-9,10-ethanoanthracen-9-yl)propyl]-amine (EP Impurity B).

³ 3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)propan-1-amine (EP Impurity C).

⁴ 3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-N-methylprop-2-en-1-amine (EP Impurity D).

⁵ 3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-N,N-dimethylpropan-1-amine (EP Impurity E).

Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unknown impurity	—	0.10
Total impurities	—	1.0

¹ 3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)acrylaldehyde (EP Impurity A).

² N-Methyl-N,N-bis[3-(9,10-dihydro-9,10-ethanoanthracen-9-yl)propyl]-amine (EP Impurity B).

³ 3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)propan-1-amine (EP Impurity C).

⁴ 3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-N-methylprop-2-en-1-amine (EP Impurity D).

⁵ 3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-N,N-dimethylpropan-1-amine (EP Impurity E).

SPECIFIC TESTS

• LOSS ON DRYING (731)

Sample: Dry a sample under vacuum at 80° to constant weight.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• USP REFERENCE STANDARDS (11)

USP Maprotiline Hydrochloride RS

USP Maprotiline Related Compound D RS

3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-N-methylprop-2-en-1-amine.

C₂₀H₂₂N 276.4

Maprotiline Hydrochloride Tablets

DEFINITION

Maprotiline Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of maprotiline hydrochloride (C₂₀H₂₃N · HCl).

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHY

Standard solution: 20 mg/mL of USP Maprotiline Hydrochloride RS in methanol

Sample solution: Transfer a portion of powdered Tablets, equivalent to 100 mg of maprotiline hydrochloride, to a glass-stoppered centrifuge tube. Add 5.0 mL of methanol to the tube, sonicate for 10 min, shake by mechanical means for 10 min, and centrifuge.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel that has been prewashed with chloroform by allowing chloroform to travel the full length of the plate, and dried at 100° for 30 min

Application volume: 5 µL

Developing solvent system: Secondary butyl alcohol, ethyl acetate, and 2 N ammonium hydroxide (6:3:1)

Analysis

Samples: *Standard solution* and *Sample solution*

In a suitable chromatographic chamber, place a volume of the *Developing solvent system* sufficient to develop a chromatogram. Place a beaker containing 25 mL of ammonium hydroxide in the bottom of the chamber, and allow it to equilibrate for 1 h. Apply *Samples* and allow the spots to dry. Develop the chromatograms until the solvent front has moved three-fourths of the length of the plate, remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Expose the

plate to hydrogen chloride vapor for 30 min, and expose it to a high-intensity UV light irradiator (1000 to 1600 watts) for 5 min. **[CAUTION—UV irradiators emit UV radiation that is harmful to eyes and skin.]** Compare the chromatograms under long-wavelength UV light.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

- **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Mobile phase: Dissolve 4 g of tetramethylammonium chloride in 495 mL of water, and add 500 mL of acetonitrile and 1 mL of phosphoric acid.

Standard solution: 0.75 mg/mL of USP Maprotiline Hydrochloride RS prepared as follows. Transfer a suitable quantity of the USP Maprotiline Hydrochloride RS to a suitable volumetric flask. Add 10% of the flask volume each of methanol and 0.1 N hydrochloric acid. Sonicate for 5 min, and then dilute to volume with water.

Sample stock solution: Transfer NLT 15 Tablets to a 500-mL volumetric flask, add 100 mL of 0.1 N hydrochloric acid, sonicate, and shake occasionally for 5 min to disintegrate the Tablets. Add 100 mL of methanol, shake, and sonicate for 5 min. Dilute with water to volume, and centrifuge. Use the supernatant.

Sample solution: Nominally 0.75 mg/mL of maprotiline hydrochloride from *Sample stock solution* and water.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 272 nm

Column: 8-mm × 10-cm; 10-μm packing L10

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of maprotiline hydrochloride ($C_{20}H_{23}N \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of maprotiline from the *Sample solution*

r_s = peak response of maprotiline from the *Standard solution*

C_s = concentration of USP Maprotiline Hydrochloride RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of maprotiline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: Dilute hydrochloric acid (7 in 1000); 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Standard solution: USP Maprotiline Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* (711).

Instrumental conditions

Mode: UV

Analytical wavelengths: About 268 nm absorbance minimum; about 272 nm absorbance maximum

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of maprotiline hydrochloride ($C_{20}H_{23}N \cdot HCl$) dissolved from UV absorbances, using the difference between the absorbance maximum and the absorbance minimum of filtered portions of the *Sample solution*, suitably diluted with *Medium*, in comparison with the *Standard solution* having a known concentration of USP Maprotiline Hydrochloride RS.

Tolerances: NLT 75% (Q) of the labeled amount of maprotiline hydrochloride ($C_{20}H_{23}N \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS** (11)
USP Maprotiline Hydrochloride RS

Marbofloxacin Compounded Oral Suspension, Veterinary

DEFINITION

Marbofloxacin Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of marbofloxacin ($C_{17}H_{19}FN_4O_4$).

Prepare Marbofloxacin Compounded Oral Suspension, Veterinary 25 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Marbofloxacin powder	2.5 g
Purified Water	A small amount
Vehicle: 1:1 mixture of Ora-Plus ^a and Ora-Sweet ^a ; or Ora-Blend ^a , a sufficient quantity to make	100 mL

^aPerrigo Pharmaceuticals, Allegan, MI.

Wet the *Marbofloxacin powder* with a small amount of *Purified Water* and triturate to make a smooth paste. Add the *Vehicle* to make the mortar contents pourable. Transfer the contents of the mortar stepwise and quantitatively to a calibrated container using the *Vehicle*. Add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY

PROCEDURE

Solution A: 10 mM ammonium formate containing 0.1% (v/v) formic acid

Solution B: Methanol containing 0.1% formic acid

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
0.5	90	10
23.0	70	30
23.5	70	30
23.6	0	100
26	0	100

Table 1 (Continued)

Time (min)	Solution A (%)	Solution B (%)
26.1	90	10
30	90	10

Standard solution: 0.4 mg/mL of marbofloxacin in Solution A

Sample solution: Transfer 1.6 mL of Oral Suspension, Veterinary to a 100-mL volumetric flask, dilute with Solution A to volume, and mix well. Pass through a PVDF filter of 0.2- μ m pore size.

Chromatographic system
(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV-Vis 327 nm

Columns

Guard: Packing L1

Analytical: 4.6-mm \times 15-cm; 5- μ m packing L1

Column temperature: 50°

Flow rate: 1.5 mL/min

Injection volume: 2 μ L

System suitability

Sample: Standard solution

[NOTE—The retention time for marbofloxacin is about 6.2 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of marbofloxacin ($C_{17}H_{19}FN_4O_4$) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of marbofloxacin from the Sample solution

r_S = peak response of marbofloxacin from the Standard solution

C_S = concentration of marbofloxacin in the Standard solution (mg/mL)

C_U = nominal concentration of marbofloxacin in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

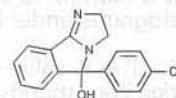
SPECIFIC TESTS

- PH (791):** 6.2–7.2

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Package in tight, light-resistant, plastic containers. Store in a refrigerator (2°–8°) or at controlled room temperature.
- BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored in a refrigerator (2°–8°) or at controlled room temperature. [NOTE—A slight darkening in yellow color may occur in the suspension with 1:1 Ora-Plus and Ora-Sweet that does not affect the strength of the preparation.]
- LABELING:** Label it to indicate that it is for veterinary use only. Label it to indicate that it is to be well shaken before use, and to state the Beyond-Use Date.

Mazindol



$C_{16}H_{13}ClN_2O$ 284.74

3H-imidazo[2,1-a]isoindol-5-ol, 5-(4-chlorophenyl)-2,5-dihydro-, (±)-

(±)-5-(p-Chlorophenyl)-2,5-dihydro-3H-imidazo[2,1-a]isoindol-5-ol [22232-71-9].

» Mazindol contains not less than 98.0 percent and not more than 102.0 percent of $C_{16}H_{13}ClN_2O$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Mazindol RS

Clarity and color of solution—A 1 in 100 solution of Mazindol in a mixture of chloroform and methanol (9:1) is clear and not darker in color than a solution prepared by mixing equal volumes of Matching Fluid C (see Color and Achromicity <631>) and water.

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 10 μ g per mL.

Medium: 0.6 N hydrochloric acid.

Absorptivities at 272 nm, calculated on the dried basis, do not differ by more than 3.0%.

Loss on drying (731)—Dry it in vacuum at 60° for 2 hours; it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals, Method II (231):** 0.002%. • (Official 1-Jan-2018)

Sulfate (221)—Triturate a 500-mg portion with 10 mL of water in a mortar. Filter the suspension through a water-washed filter, and rinse the mortar and filter with 30 mL of water, collecting the combined filtrate and washings in a 50-mL color-comparison tube. The filtrate shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.04%).

Chromatographic purity—Dissolve 10 mg in 2.0 mL of a mixture of chloroform and methanol (9:1) to obtain the test solution. Dissolve a suitable quantity of USP Mazindol RS in a mixture of chloroform and methanol (9:1) to obtain a Standard solution having a concentration of 5.0 mg per mL. Dilute portions of this solution quantitatively and stepwise with the mixture of chloroform and methanol (9:1) to obtain a series of diluted standard solutions having concentrations of 0.100, 0.050, 0.025, and 0.0125 mg per mL, respectively. Separately apply a 20- μ L portion of the test solution and 20- μ L portions of the Standard solution and each diluted standard solution to a suitable thin-layer chromatographic plate (see Chromatography <621>) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, alcohol, and ammonium hydroxide (80:20:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wave-length UV light: the chromatograms show principal spots at about the same R_f value. Estimate the concentration of any

secondary spots present in the chromatogram from the test solution by comparison with the diluted standard solutions: the principal spots from the 0.100, 0.050, 0.025, and 0.0125 mg per mL dilutions are equivalent to 2.0%, 1.0%, 0.50%, and 0.25% of impurities, respectively. No individual impurity is greater than 1.0%, and the sum of the impurities is not greater than 2.0%.

Assay—Transfer about 230 mg of Mazindol, accurately weighed, to a suitable flask, dissolve in 40 mL of glacial acetic acid, add 3 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to an emerald-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 28.47 mg of $C_{16}H_{13}ClN_2O$.

Mazindol Tablets

» Mazindol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mazindol ($C_{16}H_{13}ClN_2O$).

Packaging and storage—Preserve in tight containers, at a temperature not exceeding 25°.

USP Reference standards (11)—
USP Mazindol RS

Identification—Place a portion of powdered Tablets, equivalent to about 1 mg of mazindol, in a suitable flask. Add 40 mL of methanol, shake by mechanical means for not less than 5 minutes, and heat for several minutes on a steam bath to boiling. Cool, dilute with methanol to about 100 mL, and filter. Separate the filtrate into two approximately equal portions, add 2 drops of hydrochloric acid to one portion, and mix: the UV absorption spectra of the solutions so obtained exhibit maxima and minima at the same wavelengths as those of similar solutions prepared from USP Mazindol RS, concomitantly measured.

Dissolution (711)—

Medium: 0.01 N hydrochloric acid; 500 mL.

Apparatus 2: 50 rpm.

Time: 120 minutes.

Determine the amount of $C_{16}H_{13}ClN_2O$ dissolved by employing the following method.

Mobile phase—Mix 11.50 g of monobasic ammonium phosphate and 1.32 g of dibasic ammonium phosphate with water to obtain 1000 mL of an ammonium phosphate buffer. The *Mobile phase* is a suitably filtered and degassed mixture of the ammonium phosphate buffer and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 271-nm detector and a 4-mm × 30-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph three replicate injections of the Standard solution, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 3.0%.

Procedure—Inject an appropriate volume (50 µL to 500 µL) of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the quantity of $C_{16}H_{13}ClN_2O$ dissolved in comparison with a Standard solution having a known concentration of USP Mazindol RS in the same *Medium* and similarly chromatographed.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{16}H_{13}ClN_2O$ is dissolved in 120 minutes.

Uniformity of dosage units (905): meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

Dye solution—Dissolve 100 mg of bromocresol purple in 1000 mL of 0.33 N acetic acid, and mix.

Standard solution—Dissolve an accurately weighed quantity of USP Mazindol RS in 0.33 N acetic acid, and dilute quantitatively and stepwise with 0.33 N acetic acid to obtain a solution having a known concentration of about 20 µg per mL.

Test solution—Mix 1 finely powdered Tablet with an accurately measured volume of 0.33 N acetic acid, sufficient to provide a solution having a concentration of about 20 µg of mazindol per mL, shake by mechanical means for 30 minutes, and filter, discarding the first few mL of the filtrate.

Procedure—Transfer 25.0 mL each of the *Standard solution*, the *Test solution*, and 0.33 N acetic acid to provide the blank, to individual 125-mL separators. Add 30 mL of *Dye solution* and 50.0 mL of chloroform to each, and shake by mechanical means for 15 minutes. Allow the layers to separate, and filter the chloroform layers. Concomitantly determine the absorbances of the filtered solutions obtained from the *Test solution* and the *Standard solution* at the wavelength of maximum absorbance at about 420 nm, using the blank to set the instrument. Calculate the quantity, in mg, of mazindol ($C_{16}H_{13}ClN_2O$) in the Tablet by the formula:

$$(TC / D)(A_U / A_S)$$

in which *T* is the labeled quantity, in mg, of mazindol in the Tablet; *C* is the concentration, in µg per mL, of USP Mazindol RS in the *Standard solution*; *D* is the concentration, in µg per mL, of mazindol in the *Test solution*, based on the labeled quantity per Tablet and the extent of dilution; and *A_U* and *A_S* are the absorbances of the solutions from the *Test solution* and the *Standard solution*, respectively.

Assay—

Internal standard solution—Dissolve 50 mg of amitriptyline hydrochloride in 250 mL of methanol, and mix.

Standard preparation—Transfer about 32 mg of USP Mazindol RS, accurately weighed, to a 100-mL volumetric flask, add about 50 mL of *Internal standard solution*, and shake by mechanical means for 30 minutes. Dilute with *Internal standard solution* to volume, and mix.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 8 mg of mazindol, to a suitable flask, add 25.0 mL of *Internal standard solution*, and shake by mechanical means for 30 minutes. Pass through a fine-porosity, sintered-glass filter, discarding the first few mL of the filtrate.

Mobile phase—Transfer 200 mL of aqueous 0.01 M dibasic ammonium phosphate to a 1000-mL volumetric flask, dilute with methanol to volume, and mix. Pass through a 0.5-µm porosity polytetrafluoroethylene filter, and degas under vacuum. Protect this solution from light.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L10. Inject three replicate portions of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, is not less than 2.0; and the relative standard deviation is not more than 3.0%.

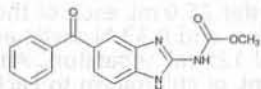
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for mazindol and amitriptyline hydrochloride. Calculate the quantity, in mg, of mazindol

(C₁₆H₁₃ClN₂O) in the portion of Tablets taken by the formula:

$$25C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Mazindol RS in the *Standard preparation*; and R_U and R_S are the peak response ratios of mazindol to amitriptyline hydrochloride obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Mebendazole



C₁₆H₁₃N₃O₃ 295.29
Carbamic acid, (5-benzoyl-1H-benzimidazol-2-yl), methyl ester;
Methyl 5-benzoyl-2-benzimidazolecarbamate [31431-39-7].

DEFINITION

Mebendazole contains NLT 98.0% and NMT 102.0% of mebendazole (C₁₆H₁₃N₃O₃), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Solution A: 7.5 g/L of ammonium acetate

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
15	70	30
20	10	90
25	10	90
26	80	20
30	80	20

Diluent: Acetonitrile and water (50:50)

System suitability solution: 50 µg/mL of USP Mebendazole RS and 2.5 µg/mL of USP Mebendazole Related Compound D RS in *Diluent*. Sonicate in *Diluent* using 80% of the final volume at 45°–50° for 10 min. Dilute with *Diluent* to final volume.

Standard solution: 0.05 mg/mL of USP Mebendazole RS in *Diluent*. Sonicate in *Diluent* using 80% of the final volume at 45°–50° for 10 min. Dilute with *Diluent* to final volume.

Sample solution: 0.05 mg/mL of Mebendazole in *Diluent*. Sonicate in *Diluent* using 80% of the final volume at 45°–50° for 10 min. Dilute with *Diluent* to final volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times of mebendazole and mebendazole related compound D are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 5.0 between mebendazole and mebendazole related compound D, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mebendazole (C₁₆H₁₃N₃O₃) in the portion of Mebendazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mebendazole from the *Sample solution*

r_S = peak response of mebendazole from the *Standard solution*

C_S = concentration of USP Mebendazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Mebendazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS**, Method II (231): NMT 20 ppm (Official 1: Jan-2018)

• ORGANIC IMPURITIES

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Prepare as directed in the *Assay*.

Standard solution: 2.5 µg/mL of USP Mebendazole RS in *Diluent*

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 5.0 between the mebendazole and mebendazole related compound D peaks

Relative standard deviation: NMT 1.0% for the mebendazole peak and NMT 5.0% for the mebendazole related compound D peak

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Mebendazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of mebendazole from the *Standard solution*

C_S = concentration of USP Mebendazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Mebendazole in the *Sample solution* (mg/mL)

F = relative response factor (see Table 2)

Acceptance criteria: See Table 2. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Amino mebendazole ^a	0.46	1.0	0.25
2-Hydroxy mebendazole ^b	0.53	1.0	0.25
2-Amino-1-methyl mebendazole ^c	0.67	1.0	0.25
Mebendazole	1.0	—	—
Mebendazole related compound D	1.1	1.0	0.25
Ethyl mebendazole ^d	1.3	1.0	0.25
Toluoyl mebendazole ^e	1.4	1.0	0.25
Mebendazole dimer ^f	1.6	0.71	0.5
Any other unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

^a 2-Amino-5-benzoylbenzimidazole.

^b 5-Benzoyl-2-hydroxybenzimidazole.

^c 2-Amino-5-benzoyl-1-methylbenzimidazole.

^d Ethyl 5-benzoyl-1-methylbenzimidazol-2-ylcarbamate.

^e Methyl 5-(4-toluoyl)-1-methylbenzimidazol-2-ylcarbamate.

^f 1,3-Bis(5-benzoylbenzimidazol-2-yl)urea.

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• USP REFERENCE STANDARDS (11)

USP Mebendazole RS

USP Mebendazole Related Compound D RS

Methyl 5-benzoyl-1-methylbenzimidazol-2-ylcarbamate.

C₁₇H₁₅N₃O₃ 309.32

Mebendazole Oral Suspension

» Mebendazole Oral Suspension is Mebendazole in an aqueous vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mebendazole (C₁₆H₁₃N₃O₃).

Packaging and storage—Preserve in tight containers at controlled room temperature.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Mebendazole RS

Identification—Mix a quantity of Oral Suspension, equivalent to about 200 mg of mebendazole, with 20 mL of a mixture of chloroform and 96 percent formic acid (19:1). Proceed as directed for Identification under Mebendazole Tablets, beginning with "Warm the suspension on a water bath for a few minutes." The specified result is obtained.

pH (791): between 6.0 and 7.0.

Assay—

Standard preparation—Transfer about 10 mg of USP Mebendazole RS, accurately weighed, to a 100-mL volumetric flask, and add 90 mL of chloroform, 7 mL of isopropyl alcohol, and 2 mL of 96 percent formic acid. Agitate until the solid has dissolved, add isopropyl alcohol to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with isopropyl alcohol to volume, and mix to obtain a solution having a known concentration of about 5 µg per mL.

Assay preparation 1—Transfer an accurately measured quantity of Oral Suspension, equivalent to about 1000 mg of mebendazole, to a 100-mL volumetric flask, dilute with 96 percent formic acid to volume, and mix. Transfer 10.0 mL of this mixture to a second 100-mL volumetric flask, add 40 mL of 96 percent formic acid, and heat in a water bath at a temperature of 50° for 15 minutes. Cool, add water to volume, mix, and pass through a medium-porosity, sintered-glass filter. Transfer 10.0 mL of the filtrate to a 250-mL separator, and add 50 mL of water and 50 mL of chloroform. Shake for about 2 minutes, allow the phases to separate, and transfer the chloroform layer to a second 250-mL separator. Wash the aqueous layer with two 10-mL portions of chloroform, add the chloroform washings to the second separator, and discard the aqueous layer. Wash the combined chloroform solutions with a mixture of 4 mL of 1 N hydrochloric acid and 50 mL of a 1 in 10 solution of 96 percent formic acid in water, and transfer the chloroform layer to a 100-mL volumetric flask. Extract the aqueous washing with two 10-mL portions of chloroform, add these chloroform extracts to the chloroform solution in the volumetric flask, add 2 mL of 96 percent formic acid and 7 mL of isopropyl alcohol, dilute with chloroform to volume, and mix. Transfer 5.0 mL of this solution to another 100-mL volumetric flask, dilute with isopropyl alcohol to volume, and mix.

Assay preparation 2 (where the Oral Suspension is packaged in syringes calibrated to deliver stated increments of mebendazole)—Express an increment of Oral Suspension to a volumetric flask of an appropriate nominal volume so that when diluted with 96 percent formic acid to volume a mixture containing about 10 mg of mebendazole per mL is obtained. Transfer 10.0 mL of this mixture to a 100-mL volumetric flask, add 40 mL of 96 percent formic acid, and heat in a water bath at a temperature of 50° for 15 minutes. Proceed as directed for Assay preparation 1 beginning with "Cool, add water to volume."

Procedure—Mix 90 mL of chloroform with 2 mL of 96 percent formic acid in a 100-mL volumetric flask, add isopropyl alcohol to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with isopropyl alcohol to volume, and mix to obtain a reagent blank. Concomitantly determine the absorbances of the relevant Assay preparation and the Standard preparation at the wavelength of maximum absorbance at about 247 nm with a spectrophotometer, using the reagent blank to set the instrument. Calculate the quantity, in mg, of mebendazole (C₁₆H₁₃N₃O₃) in the portion of Oral Suspension taken to prepare Assay preparation 1 by the formula:

$$200C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Mebendazole RS in the Standard preparation; and A_U and A_S are the absorbances of Assay preparation 1 and the Standard preparation, respectively. Where appropriate, calculate the quantity, in mg, of mebendazole (C₁₆H₁₃N₃O₃) in the incre-

ment of Oral Suspension taken to prepare Assay preparation 2 by the formula:

$$20,000(C/V)(A_U/A_S)$$

in which V is the volume, in mL, of the volumetric flask into which the increment of Oral Suspension was expressed; A_U is the absorbance of Assay preparation 2; and the other terms are as defined above.

Mebendazole Tablets

DEFINITION

Mebendazole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of mebendazole ($C_{16}H_{13}N_3O_3$).

IDENTIFICATION

• A.

Standard solution: 10 mg/mL of USP Mebendazole RS in chloroform and 96% formic acid (19:1)

Sample solution: Finely powder a quantity of Tablets, equivalent to 200 mg of mebendazole, and mix the powder with 20 mL of a mixture of chloroform and 96% formic acid (19:1). Warm the suspension on a water bath for a few min. Cool, and pass through a medium-porosity, sintered-glass filter.

Chromatographic system

(See Chromatography <621>, Thin-Layer Chromatography.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: Chloroform, methanol, and 96% formic acid (90:5:5)

Analysis

Samples: Standard solution and Sample solution

Apply the Samples to the plate, and allow the spots to dry. Develop the chromatogram in the Developing solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength UV light.

Acceptance criteria: The R_f value of the principal spot of the Sample solution corresponds to that of the Standard solution.

ASSAY

• PROCEDURE

Solution A: 0.05 M monobasic potassium phosphate

Mobile phase: Methanol and Solution A (60:40). Adjust with 0.1 M phosphoric acid or 1 N sodium hydroxide to a pH of 5.5, and filter.

Standard stock solution: 0.25 mg/mL of USP Mebendazole RS prepared as follows. Transfer 25 mg of USP Mebendazole RS into a 100-mL volumetric flask. Add 10 mL of formic acid, and heat in a water bath at 50° for 15 min. Shake by mechanical means for 5 min, add 90 mL of methanol, and allow to cool. Dilute with methanol to volume.

Standard solution: 0.05 mg/mL of USP Mebendazole RS in Mobile phase from the Standard stock solution

Sample stock solution: Nominally 0.25 mg/mL of mebendazole prepared as follows. Transfer an equivalent to 500 mg of mebendazole, from finely powdered Tablets (NLT 20), to a 100-mL volumetric flask. Add 50 mL of formic acid, and heat in a water bath at 50° for 15 min. Shake by mechanical means for 1 h, dilute with water to volume, mix, and filter. Transfer 5.0 mL of the filtrate to a 100-mL volumetric flask, and dilute with a solution of formic acid in methanol (1:9) to volume.

Sample solution: Nominally 0.05 mg/mL of mebendazole in Mobile phase from the Sample stock solution. Pass the solution through a suitable filter of 0.5- μ m pore size.

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV 247 nm

Columns

Precolumn: Contains packing L1

Analytical column: 3.9-mm \times 30-cm; packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 15 μ L

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 2500 theoretical plates

Relative standard deviation: NMT 1%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of mebendazole ($C_{16}H_{13}N_3O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mebendazole from the Sample solution

r_S = peak response of mebendazole from the Standard solution

C_S = concentration of USP Mebendazole RS in the Standard solution (mg/mL)

C_U = nominal concentration of mebendazole in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid containing 1.0% sodium lauryl sulfate; 900 mL

Apparatus 2: 75 rpm

Time: 120 min

Solution A: Dissolve 8.0 g of sodium hydroxide in 2 L of water. Add 3.0 g of sodium lauryl sulfate, and mix. Add 20 mL of phosphoric acid, and adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and Solution A (3:7)

Standard solution: 0.5 mg/mL of USP Mebendazole RS prepared as follows. Transfer the appropriate amount of USP Mebendazole RS to a volumetric flask. Add 20% of the final volume of formic acid, and dissolve. Dilute with methanol to volume. Dilute a portion of this solution with Medium to obtain a solution having a known concentration similar to the expected concentration in the solution under test.

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 3-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: Standard solution

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis: Determine the percentage of mebendazole ($C_{16}H_{13}N_3O_3$) dissolved.

Tolerances: NLT 75% (Q) of the labeled amount of mebendazole ($C_{16}H_{13}N_3O_3$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Standard stock solution: Transfer 20 mg of USP Mebendazole RS into a 10-mL volumetric flask. Add 4 mL

of 96% formic acid, and mix to dissolve. Add isopropyl alcohol to volume.

Standard solution: 0.01 mg/mL of USP Mebendazole RS in isopropyl alcohol, from the *Standard stock solution*

Sample solution: Mix 1 Tablet with 20 mL of 96% formic acid in a 100-mL volumetric flask, and heat on a steam bath for 15 min. Cool, add isopropyl alcohol to volume, mix, and pass through a medium pore size, sintered-glass filter. Transfer an equivalent to 1 mg of mebendazole from the filtrate to a 100-mL volumetric flask, and dilute with isopropyl alcohol to volume.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: Absorption maximum at about 310 nm

Cell length: 1 cm

Blank: 1-in-500 solution of 96% formic acid in isopropyl alcohol

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in mg, of mebendazole ($C_{16}H_{13}N_3O_3$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times L \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Mebendazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mebendazole in the *Sample solution* (mg/mL)

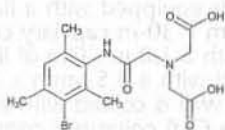
L = label claim (mg/Tablet)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
USP Mebendazole RS

Mebrofenin



$C_{15}H_{19}BrN_2O_5$ 387.23

Glycine, *N*-[2-[(3-bromo-2,4,6-trimethylphenyl)amino]-2-oxoethyl]-*N*-(carboxymethyl)-;

[[[(3-Bromomesityl)carbonyl]methyl]imino]diacetic acid [78266-06-5].

DEFINITION

Mebrofenin contains NLT 97.0% and NMT 101.0% of mebrofenin ($C_{15}H_{19}BrN_2O_5$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

ASSAY

- **PROCEDURE**

Sample solution: Dissolve 100 mg of Mebrofenin in 40 mL of dimethylformamide in a conical flask with the aid of sonication if necessary. Add 3 drops of thymol blue TS.

Analysis: Titrate the *Sample solution* with 0.1 N sodium methoxide (in toluene) VS to a blue endpoint while flushing the flask with a gentle stream of nitrogen. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 19.36 mg of mebrofenin ($C_{15}H_{19}BrN_2O_5$).

Acceptance criteria: 97.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 30 ppm (Official 1-

Jan-2018)

- **LIMIT OF NITRILOTRIACETIC ACID**

Mobile phase: Add 10 mL of a solution (1 in 4) of tetrabutylammonium hydroxide in methanol to 200 mL of water, and adjust with 1 M phosphoric acid to a pH of 7.5 ± 0.1 . Transfer this solution to a 1000-mL volumetric flask, add 90 mL of methanol, and dilute with water to volume.

Diluent: 10 mg/mL of cupric nitrate solution

Standard stock solution: 0.5 mg/mL of nitrilotriacetic acid in dilute ammonium hydroxide (1 in 20)

Standard solution: 0.05 mg/mL of nitrilotriacetic acid from a suitable volume of *Standard stock solution* in *Diluent*. [NOTE—Prepare fresh on the day of use.]

Sample solution: 10 mg/mL of Mebrofenin in *Diluent*. Sonicate, if necessary, to dissolve. [NOTE—Prepare fresh on the day of use.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; packing L7

Flow rate: 0.8 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[NOTE—Peaks containing copper may be present.]

Suitability requirements

Resolution: NLT 1.7 between the major peak and any other peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nitrilotriacetic acid in the portion of Mebrofenin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of copper nitrilotriacetic acid from the *Sample solution*

r_S = peak response of copper nitrilotriacetic acid from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1%

- **ORGANIC IMPURITIES**

Buffer: Prepare a mixture of equal volumes of 0.025 M monobasic potassium phosphate and 0.025 M dibasic sodium phosphate.

Mobile phase: Mix 400 mL of *Buffer* with 600 mL of methanol in a 1-L volumetric flask. Dilute with water to volume. Adjust with 1 N hydrochloric acid to a pH of 5.0 ± 0.1 .

Sample solution: 0.5 mg/mL of Mebrofenin in *Mobile phase*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 220 nm**Column:** 4.6-mm × 25-cm; packing L1**Flow rate:** 1 mL/min**Injection volume:** 20 µL**Run time:** Twice the retention time of mebrofenin**System suitability****Sample:** *Sample solution***Suitability requirements****Capacity factor, *k'*:** NLT 1.2**Column efficiency:** NLT 200 plates**Tailing factor:** NMT 4.0**Relative standard deviation:** NMT 2.0% for the mebrofenin peak**Analysis****Sample:** *Sample solution*

Calculate the percentage of impurities in the portion of Mebrofenin taken:

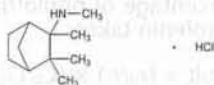
$$\text{Result} = (r_u/r_r) \times 100$$

r_u = sum of the peak responses of the individual impurities*r_r* = total of all of the peak responses in the chromatogram**Acceptance criteria:** NMT 3%**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE, Class I (741):** 185°–200°, but the range between the beginning and end of melting does not exceed 4°.
- **LOSS ON DRYING (731)**
Analysis: Dry under vacuum at 100° for 3 h.
Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS (11)**
USP Mebrofenin RS

Mecamylamine Hydrochloride $\text{C}_{11}\text{H}_{21}\text{N} \cdot \text{HCl}$ 203.75Bicyclo[2.2.1]heptan-2-amine, *N*,2,3,3-tetramethyl-, hydrochloride.*N*,2,3,3-Tetramethyl-2-norbornanamine hydrochloride [826-39-1].

» Mecamylamine Hydrochloride contains not less than 98.0 percent and not more than 100.5 percent of $\text{C}_{11}\text{H}_{21}\text{N} \cdot \text{HCl}$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.**USP Reference standards (11)**—

USP Mecamylamine Hydrochloride RS

USP Mecamylamine Related Compound A RS

N,1,7,7-Tetramethyl bicyclo [2.2.1]heptan-2-amine. $\text{C}_{11}\text{H}_{21}\text{N}$ 167.29**Identification—****A:** *Infrared Absorption* (197K).**B:** It responds to the tests for *Chloride* (191).

Acidity—Dissolve 5.0 g in 100 mL of methanol, and titrate potentiometrically with 0.10 N alcoholic potassium hydroxide to an apparent pH of 5.5, using a calomel-glass electrode system and a potentiometer previously standardized with pH 5.0 neutralized phthalate buffer (see *Solutions* in the section *Reagents, Indicators, and Solutions*): after correction for the volume of alkali consumed by 100 mL of methanol, not more than 0.55 mL of 0.10 N alcoholic potassium hydroxide is required.

Loss on drying (731)—Dry it at a pressure not exceeding 5 mm of mercury at 105° for 1 hour: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.5%.**Delete the following:**

• **Heavy metals, Method I (231)**—Dissolve 400 mg in 20 mL of water, add 2 mL of 1 N acetic acid, and dilute with water to 25 mL: the limit is not more than 50 ppm.

(Official 1-Jan-2018)

Limit of residual solvents—**Diluent**—Prepare a mixture of dimethyl sulfoxide and water (2:1).**Internal standard solution**—Prepare a solution of absolute alcohol in *Diluent* having a known concentration of about 15 µL per mL.**Standard stock solution**—Transfer 50 mL of the *Diluent* to a 100-mL volumetric flask, add 0.64 mL of isopropyl alcohol, dilute with *Diluent* to volume, and mix.**Standard solution**—Pipet 1.0 mL of the *Standard stock solution* into a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix (*Solution 1*). Transfer about 500 mg of sodium chloride, accurately weighed, to a headspace vial, add 1.5 mL of *Solution 1* and 1.5 mL of the *Internal standard solution*, and mix.**Test solution**—Transfer about 150 mg of Mecamylamine Hydrochloride, accurately weighed, to a headspace vial, add about 500 mg of sodium chloride, 1.5 mL of *Diluent*, and 1.5 mL of the *Internal standard solution*, and mix.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m capillary column whose internal wall is coated with a 1.0-µm film of liquid phase G16. This column is joined with a 0.53-mm × 25-m capillary column whose internal wall is coated with a 5.0-µm film of liquid phase G1. The G16 column is connected to the detector, and the G1 column is connected to the injector. The injection port temperature is maintained at about 100°; the detector temperature is maintained at about 210°; and the column temperature is maintained at 50° for 10 minutes, then increased at a rate of 5° per minute to 110°, then increased at a rate of 30° per minute to 210°, and maintained for 5 minutes at 210°. Nitrogen is used as the carrier gas, flowing at a rate of about 6.5 mL per minute. The split flow is 15 mL per minute.

Procedure—Allow the *Standard solution*, the *Internal standard solution*, and the *Test solution* to stand for 20 minutes at 90°. Separately inject equal volumes (about 1 mL) of the headspace of the *Standard solution*, the *Internal standard solution*, and the *Test solution* into the gas chromatograph, record the chromatograms, and measure the peak responses of the internal standard and isopropyl alcohol. Calculate the

quantity, in ppm, of isopropyl alcohol in the portion of Mecamylamine Hydrochloride taken by the formula:

$$150(R_U)(W_S) / (R_S)(W_U)$$

in which W_S is the amount, in ppm, of isopropyl alcohol in the *Standard solution*; W_U is the weight, in mg, of Mecamylamine Hydrochloride taken to prepare the *Test solution*; and R_U and R_S are the peak response ratios of isopropyl alcohol to the internal standard obtained from the *Test solution* and the *Standard solution*, respectively: not more than 2000 ppm of isopropyl alcohol is found.

Related compounds—

Internal standard solution—Proceed as directed in the *Assay*.

Solution 1—Prepare a solution of *dl*-camphene and USP Mecamylamine Related Compound A RS in the *Internal standard solution* containing 625 µg of each per mL.

System suitability solution—Transfer about 125 mg of USP Mecamylamine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 1 mL of *Solution 1*, dilute with *Internal standard solution* to volume, and mix.

Test solution—Use the *Assay preparation*.

Chromatographic system—Prepare as directed in the *Assay*. Chromatograph the *System suitability solution*, and record peak responses as directed for *Procedure*: the resolution, R , between the mecamlamine and mecamlamine related compound A is not less than 5; the column efficiency is not less than 4000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject a volume (about 1 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all the peak responses. Calculate the percentage of each impurity in the portion of Mecamylamine Hydrochloride taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses for all the peaks: not more than 0.5% of mecamlamine related compound A is found; not more than 0.5% of *dl*-camphene is found; and not more than 1.0% of total impurities is found.

Chloride content—Dissolve about 500 mg, accurately weighed, in 5 mL of water. Add 5 mL of glacial acetic acid, 50 mL of methanol, and 1 drop of eosin Y TS, and titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl: the content is between 17.0% and 17.8%.

Assay—

Internal standard solution—Transfer about 600 mg of sodium hydroxide pellets to a 1 L volumetric flask, dissolve in about 800 mL of methanol. Add an accurately weighed quantity of about 1.7 g of biphenyl to the flask, and dilute with methanol to volume.

Standard preparation—Dissolve an accurately weighed quantity of USP Mecamylamine Hydrochloride RS in *Internal standard solution*, and dilute with *Internal standard solution*, quantitatively and stepwise if necessary, to obtain a solution having a known concentration of about 2.5 mg per mL.

Assay preparation—Transfer about 125 mg of Mecamylamine Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Internal standard solution* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector connected to a 0.53-mm × 30-m capillary column, coated with a 1.5-µm film of liquid phase G27. The injection port temperature is maintained at about 200°, the detector temperature is maintained at about 280°, and the column temperature is at 120° for 15 minutes then in-

creased at 25° per minute to 250° and maintained for 7 minutes at 250°. Nitrogen is used as the carrier gas at 7.4 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 4000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject equal volumes (about 1 µL) of the *Assay preparation* and the *Standard preparation* into the gas chromatograph, record the chromatogram, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{11}H_{21}N \cdot HCl$ in the portion of Mecamylamine Hydrochloride taken by the formula:

$$50C(R_U / R_S)$$

in which C is the concentration of USP Mecamylamine Hydrochloride RS, in mg per mL, in the *Standard preparation*; and R_U and R_S are the peak response ratios of mecamlamine hydrochloride to the internal standard biphenyl obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Mecamylamine Hydrochloride Tablets

» Mecamylamine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mecamlamine hydrochloride ($C_{11}H_{21}N \cdot HCl$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Mecamylamine Hydrochloride RS

Identification—

A: To a quantity of powdered Tablets, equivalent to about 75 mg of mecamlamine hydrochloride, add 50 mL of chloroform, and triturate the mixture for 5 minutes. Filter, and evaporate the filtrate on a steam bath with the aid of a current of air to dryness: the IR absorption spectrum of a potassium bromide dispersion of a portion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Mecamylamine Hydrochloride RS.

B: A portion of the residue obtained in *Identification test A* responds to the tests for *Chloride* (191).

Dissolution (711)—

Medium: water; 750 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{11}H_{21}N \cdot HCl$ dissolved using the following procedure.

Diluent—Prepare a solution of triethylamine in alcohol (1:100).

Internal standard solution—Prepare a solution of biphenyl in *Diluent* having a concentration of 82.5 µg per mL.

Standard solution—Prepare a solution of USP Mecamylamine Hydrochloride RS and biphenyl in *Diluent* having concentrations of 8.25 µg per mL of each.

Test solution—[NOTE—Condition the solid-phase extraction column specified in this procedure in the following manner. Wash the column with 5 mL of water, then with 5 mL of *Diluent*, and finally with two 5-mL portions of water.] Transfer by pipetting 25.0 mL of the solution under test through a freshly conditioned solid-phase extraction column containing L1 packing with a sorbent-mass to column volume ratio of 360 mg per 5 mL, or equivalent. Wash the pipet and the

solid-phase extraction column with two 5-mL portions of water. Discard the filtrate. Elute the solid-phase extraction column with two 4-mL portions of *Diluent*, and collect the eluate in a 10-mL volumetric flask containing 1.0 mL of *Internal standard solution*. Dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector, a splitless injection system, and a 0.53-mm × 30-m analytical column coated with a 1.5-μm layer of phase G27. The carrier gas is helium at a flow rate of 5.2 mL per minute. The detector and column temperatures are maintained at 250° and 150°, respectively. Chromatograph replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2, and the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 2 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount in mg, of $C_{11}H_{21}N \cdot HCl$ dissolved by the formula:

$$0.3C(R_U / R_S)$$

in which *C* is the concentration, in μg per mL, of USP Mecamylamine Hydrochloride RS in the *Standard solution*; and R_U and R_S are the peak response ratios of the mecamlamine hydrochloride peak to the internal standard peak obtained from the *Test solution* and *Standard solution*, respectively.

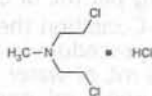
Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{11}H_{21}N \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Procedure for content uniformity—Place 1 Tablet in the digestion flask, and proceed as directed under *Nitrogen Determination, Method II* (461). Each mL of 0.01 N sulfuric acid is equivalent to 2.038 mg of mecamlamine hydrochloride.

Assay—Weigh and finely powder not fewer than 30 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of mecamlamine hydrochloride, to a glass-stoppered, 125-mL conical flask. Add about 25 mL of water, insert the stopper in the flask, and shake by mechanical means for 20 minutes. Transfer the contents of the flask to a 250-mL separator with the aid of small portions of water. Add 1 mL of 1 N sodium hydroxide and 5 g of sodium chloride, and extract the mixture successively with two 50-mL and three 25-mL portions of ether. Wash the combined ether extracts with three 10-mL portions of water, and wash, in turn, the combined water washes with a 10-mL portion of ether, adding it to the washed combined ether extracts. Transfer the ether phase to a 250-mL conical flask containing 25.0 mL of 0.02 N sulfuric acid VS, and evaporate the ether on a steam bath. Cool the solution, add methyl red TS, and titrate the excess acid with 0.02 N sodium hydroxide VS. Each mL of 0.02 N sulfuric acid is equivalent to 4.075 mg of mecamlamine hydrochloride ($C_{11}H_{21}N \cdot HCl$).

Mechlorethamine Hydrochloride



$C_5H_{11}Cl_2N \cdot HCl$ 192.51

Ethanamine, 2-chloro-*N*-(2-chloroethyl)-*N*-methyl-, hydrochloride.

2,2'-Dichloro-*N*-methyldiethylamine hydrochloride [55-86-7].

» Mechlorethamine Hydrochloride contains not less than 97.5 percent and not more than 100.5 percent of $C_5H_{11}Cl_2N \cdot HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—The label bears a warning that great care should be taken to prevent inhaling particles of Mechlorethamine Hydrochloride and exposing the skin to it.

USP Reference standards (11)—

USP Mechlorethamine Hydrochloride RS

Identification—

A: Infrared Absorption (197K).

B: Transfer 100 mg to a test tube containing 1 mL of sodium thiosulfate solution (prepared by dissolving 1 g of sodium thiosulfate and 100 mg of sodium carbonate in 40 mL of water), shake, allow to stand for 2 hours, then add 1 drop of iodine TS: the color of free iodine remains.

Melting range (741): between 108° and 111°.

pH (791): between 3.0 and 5.0, in a solution (1 in 500).

Water Determination, Method I (921): not more than 0.4%.

Ionic chloride content—Dissolve about 30 mg, accurately weighed, in 30 mL of water contained in a beaker. Add 5 mL of nitric acid and stir. Titrate with 0.02 N silver nitrate VS to a potentiometric endpoint, using a silver combination electrode. Perform a blank determination (see *Titrimetry* (541)), and make any necessary correction. Each mL of 0.02 N silver nitrate is equivalent to 0.709 mg of ionic chloride: not less than 18.0% and not more than 19.3% of ionic chloride is found.

Assay—Transfer about 100 mg of Mechlorethamine Hydrochloride, accurately weighed, to a 125-mL conical flask. Add 100 mg of sodium bicarbonate and 20.0 mL of 0.1 N sodium thiosulfate VS. Allow to stand for 2½ hours, add 3 mL of starch TS, and titrate the excess sodium thiosulfate with 0.1 N iodine VS. Each mL of 0.1 N sodium thiosulfate is equivalent to 9.626 mg of $C_5H_{11}Cl_2N \cdot HCl$.

Mechlorethamine Hydrochloride for Injection

» Mechlorethamine Hydrochloride for Injection is a sterile mixture of Mechlorethamine Hydrochloride with Sodium Chloride or other suitable diluent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mechlorethamine hydrochloride ($C_5H_{11}Cl_2N \cdot HCl$).

Change to read:

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

Labeling—It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. The label bears a warning that great care should be taken to prevent inhaling particles of Mechlorethamine Hydrochloride for Injection and exposing the skin to it.

USP Reference standards (11)—

USP Endotoxin RS

USP Mechlorethamine Hydrochloride RS

Completeness of solution (641)—A 0.10-g portion dissolves in 10 mL of carbon dioxide-free water to yield a clear solution.

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.

Identification—It meets the requirements of the *Identification tests* under *Mechlorethamine Hydrochloride*.

Bacterial Endotoxins Test (85)—It contains not more than 12.5 USP Endotoxin Units per mg of mechlorethamine hydrochloride.

pH (791): between 3.0 and 5.0, in a solution (1 in 50).

Water Determination, Method I (921): not more than 1.0%.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Other requirements—It meets the requirements for *Sterility Tests* (71) and *Uniformity of Dosage Units* (905).

Assay—

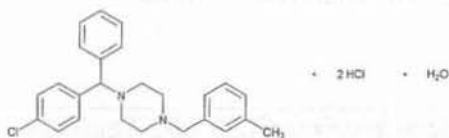
Assay preparation—Select a counted number of not fewer than 10 containers of Mechlorethamine Hydrochloride for Injection, equivalent to about 100 mg of mechlorethamine hydrochloride. Dissolve the contents of each container in water, and transfer the resulting solutions to a 250-mL conical flask.

Procedure—Immediately proceed as directed in the *Assay* under *Mechlorethamine Hydrochloride*, beginning with "Add 100 mg of sodium bicarbonate." Calculate the average content, in mg, of mechlorethamine hydrochloride ($C_5H_{11}Cl_2N \cdot HCl$) per container of Mechlorethamine Hydrochloride for Injection taken by the formula:

$$9.626(V/N)$$

in which *V* is the volume, in mL, of 0.1 N sodium thiosulfate consumed; and *N* is the number of containers selected to prepare the *Assay preparation*.

Meclizine Hydrochloride



$C_{25}H_{27}ClN_2 \cdot 2HCl \cdot H_2O$ 481.89

$C_{25}H_{27}ClN_2 \cdot 2HCl$ 463.88

Piperazine, 1-[(4-chlorophenyl)phenylmethyl]-4-[(3-methylphenyl)methyl]-, dihydrochloride, monohydrate; 1-(*p*-chloro- α -phenylbenzyl)-4-(*m*-methylbenzyl)piperazine dihydrochloride monohydrate [31884-77-2].

Anhydrous [1104-22-9].

DEFINITION

Meclizine Hydrochloride contains NLT 97.0% and NMT 102.0% of $C_{25}H_{27}ClN_2 \cdot 2HCl$, calculated on the anhydrous basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

• **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191)

Sample solution: Dissolve 25 mg in a mixture of 3 mL of 2 N nitric acid and 5 mL of alcohol.

Acceptance criteria: Meets the requirements

ASSAY• **PROCEDURE**

Mobile phase: Dissolve 1.5 g of sodium 1-heptanesulfonate in 300 mL of water, and mix this solution with 700 mL of acetonitrile. Adjust with 0.1 N sulfuric acid to a pH of 4.

Standard solution: 0.1 mg/mL of USP Meclizine Hydrochloride RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Meclizine Hydrochloride in *Mobile phase*

• **Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1.3 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of meclizine hydrochloride ($C_{25}H_{27}ClN_2 \cdot 2HCl$) in the portion of Meclizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of meclizine from the *Sample solution*

r_S = peak response of meclizine from the *Standard solution*

C_S = concentration of USP Meclizine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Meclizine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—On the basis of the synthetic route, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* is recommended when the isomeclizine impurity may be present.]

Mobile phase: Dissolve 1.5 g of sodium 1-heptanesulfonate in 300 mL of water, and mix this solution with 700 mL of acetonitrile. Adjust with 0.1 N sulfuric acid to a pH of 4.

System suitability solution: 0.01 mg/mL each of USP Meclizine Hydrochloride RS and 4-chlorobenzophenone in *Mobile phase*

Standard solution: 2.5 μ g/mL of USP Meclizine Hydrochloride RS in *Mobile phase*

Sample solution: 0.5 mg/mL of Meclizine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1.3 mL/min

Injection size: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The elution order is meclizine, followed by 4-chlorobenzophenone.]

Suitability requirements

Resolution: NLT 2.0 between meclizine hydrochloride and 4-chlorobenzophenone, *System suitability solution*

Column efficiency: NLT 1800 theoretical plates, determined from the analyte peak, *Standard solution*
 Tailing factor: NMT 1.5 for the analyte peak, *Standard solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the *Sample solution* to elute for NLT three times the retention time of meclizine hydrochloride.

Calculate the percentage of each impurity in the portion of Meclizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of meclizine from the *Standard solution*

C_S = concentration of USP Meclizine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Meclizine Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor, 0.72 for the 4-chlorobenzophenone peak and 1.0 for all other peaks

Acceptance criteria

Any individual impurity: NMT 0.5%

Total impurities: NMT 1.0%

• ORGANIC IMPURITIES, PROCEDURE 2

Mobile phase: Dissolve 5 g of sodium 1-heptanesulfonate in 1000 mL of water, and mix 600 mL of this solution with 400 mL of acetonitrile. Adjust with 0.1 N sulfuric acid to a pH of 4.0 ± 0.1 .

System suitability solution: 2.5 µg/mL each of USP Meclizine Hydrochloride RS, USP Meclizine Related Compound A RS, and USP Meclizine Related Compound B RS in *Mobile phase*

Standard solution: 2.5 µg/mL of USP Meclizine Hydrochloride RS in *Mobile phase*

Sample solution: 0.5 mg/mL of Meclizine Hydrochloride in *Mobile phase*. [NOTE—Store this solution no longer than 24 h.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 25-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 2.0 mL/min

Injection size: 30 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between meclizine related compound B and meclizine, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 6.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Meclizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of meclizine from the *Standard solution*

C_S = concentration of USP Meclizine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Meclizine Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peak eluting before 1.75 min.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
3-Methylbenzyl alcohol	0.11	1.0	0.10
1,4-Bis(3-methylbenzyl) piperazine	0.22	0.73	0.10
4-Chlorobenzhydrol ^a	0.53	1.3	0.15
Meclizine o-chloro isomer ^b	0.81	1.0	0.10
Isomeclizine (meclizine o-methyl isomer) ^c	0.90	1.1	0.15
Meclizine	1.0	—	—
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

^a USP Meclizine Related Compound A.

^b 1-[2-Chlorophenyl](phenyl)methyl]-4-(3-methylbenzyl) piperazine.

^c USP Meclizine Related Compound B.

SPECIFIC TESTS

• **WATER DETERMINATION, Method I (921):** NMT 5.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.

• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.

• **USP REFERENCE STANDARDS (11)**

USP Meclizine Hydrochloride RS

USP Meclizine Related Compound A RS

4-Chlorobenzhydrol.

$C_{13}H_{11}ClO$ 218.68

USP Meclizine Related Compound B RS

Isomeclizine

1-[(4-Chlorophenyl)(phenyl)methyl]-

4-(2-methylbenzyl)piperazine dihydrochloride monohydrate.

$C_{25}H_{27}ClN_2 \cdot 2HCl \cdot H_2O$ 481.88

Meclizine Hydrochloride Tablets

» Meclizine Hydrochloride Tablets contain not less than 95.0 percent and not more than 110.0 percent of the labeled amount of meclizine hydrochloride ($C_{25}H_{27}ClN_2 \cdot 2HCl$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Meclizine Hydrochloride RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: Thin-Layer Chromatographic Identification Test (201)—

Adsorbent: 0.5-mm layer of chromatographic silica gel mixture.

Test solution—Extract a quantity of finely powdered Tablets, equivalent to about 125 mg of meclizine hydrochloride, by shaking for 15 minutes with 50 mL of methanol.

Standard solution—Prepare a solution of USP Meclizine Hydrochloride RS in methanol, containing 2.5 mg per mL.

Application volume: 50 μ L.

Developing solvent system: a mixture of cyclohexane, toluene, and diethylamine (15:3:2).

Procedure—Proceed as directed in the chapter, except to place the plate in a developing chamber that contains and has been equilibrated with *Developing solvent system*.

Dissolution, Procedure for a Pooled Sample (711)—

Medium: 0.01 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Determine the amount of $C_{25}H_{27}ClN_2 \cdot 2HCl$ dissolved by employing the following method.

Mobile phase—Prepare a suitable degassed and filtered mixture of water and methanol (55:45) that contains 0.69 g of monobasic sodium phosphate in each 100 mL and is adjusted with phosphoric acid, if necessary, to a pH of 4.0.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm \times 25-cm analytical column that contains packing L9. The flow rate is about 2 mL per minute. Chromatograph replicate injections of the Standard solution, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

Procedure—Inject about 100 μ L of a filtered portion of the solution under test, suitably diluted with *Mobile phase*, if necessary, into the chromatograph, record the chromatogram, and measure the response for the major peak. Determine the amount of $C_{25}H_{27}ClN_2 \cdot 2HCl$ dissolved from the peak response obtained in comparison with the peak response obtained from a Standard solution having a known concentration of USP Meclizine Hydrochloride RS in a mixture of *Medium* and *Mobile phase* (1:1), similarly chromatographed. An amount of alcohol not to exceed 1% of the total volume of the Standard solution may be used to dissolve USP Meclizine Hydrochloride RS prior to dilution.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{25}H_{27}ClN_2 \cdot 2HCl$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Procedure for content uniformity—Place 1 Tablet in a 100-mL volumetric flask, add 50 mL of dilute hydrochloric acid (1 in 100), shake by mechanical means for 30 minutes, add the dilute acid to volume, and filter, discarding the first 20 mL of the filtrate. Dilute quantitatively and stepwise with the same acid to obtain a solution having a concentration of about 15 μ g of meclizine hydrochloride per mL. Similarly, prepare a Standard solution of USP Meclizine Hydrochloride RS in dilute hydrochloric acid (1 in 100) having a known concentration of about 15 μ g per mL. Concomitantly determine the absorbances of the solution from the Tablet and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 232 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 100) as the blank. Calculate the quantity, in mg, of meclizine hydrochloride ($C_{25}H_{27}ClN_2 \cdot 2HCl$) in the Tablet taken by the formula:

$$(T/D)C(A_U/A_S)$$

in which *T* is the quantity, in mg, of meclizine hydrochloride in the Tablet; *D* is the concentration, in μ g per mL, of meclizine hydrochloride in the solution from the Tablet, on the basis of the labeled quantity per Tablet and the extent

of dilution; *C* is the concentration, in μ g per mL, of USP Meclizine Hydrochloride RS in the Standard solution; and *A_U* and *A_S* are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Related compounds—

Mobile phase and Buffer pH 7.5—Prepare as directed in the Assay.

Standard solution—Dissolve an accurately weighed quantity of USP Meclizine Hydrochloride RS in *Mobile phase*, sonicating for about 5 minutes or until the material is dissolved, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.025 mg per mL. [NOTE—This solution is stable for 72 hours when stored at controlled room temperature protected from light.]

Sensitivity solution—Dilute an aliquot of the *Standard solution* with *Diluent* to obtain a solution containing about 1.25 μ g per mL. [NOTE—Prepare this solution fresh daily.]

Test solution—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of meclizine hydrochloride based on the label claim, to a 100-mL volumetric flask. Add about 50 mL of *Mobile phase* and shake by mechanical means for not less than 30 minutes. Dilute with *Mobile phase* to volume, mix, allow to settle for about 15 minutes, and pass through a 0.45- μ m nylon filter, discarding the first 5 mL of the filtrate.

Chromatographic system (see *Chromatography* (621))—Prepare as directed in the Assay. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency, *N*, is not less than 1200 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Sensitivity solution*, and record the peak responses as directed for *Procedure*: the signal-to-noise ratio, *S/N*, is not less than 10.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard solution* and the *Test solution* into the chromatograph. Allow the *Test solution* to elute for not less than two times the retention time of meclizine hydrochloride. Record the chromatograms and measure all of the peak areas. Calculate the percentage of each impurity relative to the labeled content of meclizine hydrochloride in the portion of the Tablets taken by the formula:

$$100(1/F)(C_S/C_T)(r_i/r_S)$$

in which *F* is the relative response factor, which is equal to 0.72 for the 4-chlorobenzophenone peak eluting at a relative retention time of about 0.23 and equal to 1.0 for all other peaks; *C_T* is the concentration, in mg per mL, of meclizine hydrochloride in the *Test solution*, based on the label claim; *C_S* is the concentration, in mg per mL, of meclizine hydrochloride in the *Standard solution*; *r_i* is the peak response for each impurity obtained from the *Test solution*; and *r_S* is the response of the meclizine peak obtained from the *Standard solution*: not more than 0.5% of any individual impurity is found; and not more than 1.0% of total impurities is found. Reporting level for impurities is 0.1%.

Assay—

Buffer pH 7.5—Dissolve 1.32 g of dibasic ammonium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 7.5 \pm 0.05.

Mobile phase—Prepare a mixture of *Buffer pH 7.5*, methanol, and acetonitrile (350:325:325). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Meclizine Hydrochloride RS in *Mobile phase*, sonicating for about 5 minutes or until the material is dissolved, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known con-

centration of about 0.125 mg per mL. [NOTE—This solution is stable for 72 hours when stored at controlled room temperature protected from light.]

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 12.5 mg of mecizine hydrochloride based on the label claim, to a 100-mL volumetric flask. Add about 50 mL of *Mobile phase*, and shake by mechanical means for not less than 30 minutes. Dilute with *Mobile phase* to volume, mix, and filter through a 0.45- μ m nylon filter, discarding the first 5 mL of the filtrate.

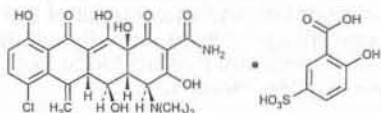
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 232-nm detector and a 4.6-mm \times 25-cm column that contains 5- μ m packing L11. The column temperature is maintained at 30°, and the flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of the labeled amount of mecizine hydrochloride ($C_{25}H_{27}ClN_2 \cdot 2HCl$) in each Tablet taken by the formula:

$$100(CV/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of mecizine hydrochloride in the *Standard preparation*; V is the volume, in mL, of the *Assay preparation*; W is the quantity, in mg, of mecizine hydrochloride based on the label claim, taken to prepare the *Assay preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Meclocycline Sulfosalicylate



$C_{22}H_{21}ClN_2O_8 \cdot C_7H_6O_6S$ 695.05
2-Naphthacenecarboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-, [4S-(4 α ,4a α ,5 α ,5a α ,12a α)]-, mono(2-hydroxy-5-sulfobenzoate) (salt).
(4S,4aR,5S,5aR,12aS)-7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacene carboxamide mono(5-sulfosalicylate) (salt) [73816-42-9].

» Meclocycline Sulfosalicylate has a potency equivalent to not less than 620 μ g of meclocycline ($C_{22}H_{21}ClN_2O_8$) per mg.

Packaging and storage—Preserve in tight containers, protected from light.

USP Reference standards (11)—

USP Meclocycline Sulfosalicylate RS

Identification, Infrared Absorption (197K).

Crystallinity (695): meets the requirements.

pH (791): between 2.5 and 3.5, in a solution containing 10 mg per mL.

Water Determination, Method I (921): not more than 4.0%.

Assay—

0.001 M Ammonium edetate—Transfer 293 mg of edetic acid, accurately weighed, to a 1000-mL volumetric flask, add 1 mL of methanol and 7 mL of ammonium hydroxide, and shake to dissolve the edetic acid. Add 900 mL of water, adjust with glacial acetic acid to a pH of 6.6, dilute with water to volume, and mix.

Mobile phase—Prepare a mixture of 0.001 M Ammonium edetate and tetrahydrofuran (85 : 15). Filter and degas the solution before use.

Standard stock preparation—Dissolve an accurately weighed quantity of USP Meclocycline Sulfosalicylate RS in methanol to obtain a solution having a known concentration of about 0.5 mg of meclocycline per mL.

Standard preparation—Immediately prior to injection, dilute the *Standard stock preparation* quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 60 μ g of meclocycline per mL.

Assay stock preparation—Transfer 36 mg of Meclocycline Sulfosalicylate, accurately weighed, to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

Assay preparation—Immediately prior to injection, transfer 3.0 mL of the *Assay stock preparation* to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution having a nominal concentration of about 60 μ g of meclocycline per mL.

Chromatographic system—The liquid chromatograph is equipped with a 340-nm detector and a 4-mm \times 25-cm column that contains packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation of the meclocycline peak for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and measure the responses for the meclocycline peak. Calculate the quantity in μ g of $C_{22}H_{21}ClN_2O_8$ in each mg of Meclocycline Sulfosalicylate taken by the formula:

$$(C_S / C_U)(r_U / r_S)$$

in which C_S is the concentration, in μ g per mL, of meclocycline in the *Standard preparation*; C_U is the concentration, in mg per mL, of Meclocycline Sulfosalicylate in the *Assay preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Meclocycline Sulfosalicylate Cream

» Meclocycline Sulfosalicylate Cream contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of meclocycline ($C_{22}H_{21}ClN_2O_8$).

Packaging and storage—Preserve in tight containers, protected from light.

USP Reference standards (11)—

USP Meclocycline Sulfosalicylate RS

Minimum fill (755): meets the requirements.

Assay—

0.001 M Ammonium edetate—Transfer 293 mg of edetic acid, accurately weighed, to a 1000-mL volumetric flask, add 1 mL of methanol and 7 mL of ammonium hydroxide, and shake to dissolve the edetic acid. Add 900 mL of water,

adjust with glacial acetic acid to a pH of 6.6, dilute with water to volume, and mix.

Mobile phase—Prepare a mixture of 0.001 M Ammonium edetate and tetrahydrofuran (85 : 15). Filter and degas the solution before use.

Standard stock preparation—Dissolve an accurately weighed quantity of USP Meclocycline Sulfosalicylate RS in methanol to obtain a solution having a known concentration of about 0.5 mg of meclocycline per mL.

Standard preparation—Immediately prior to injection, dilute the *Standard stock preparation* quantitatively, and stepwise if necessary, with *Mobile phase*, to obtain a solution having a known concentration of about 10 µg of meclocycline per mL.

Assay stock preparation—Transfer an accurately weighed quantity of Cream, equivalent to about 5 mg of meclocycline, to a glass-stoppered, 50-mL centrifuge tube. Add 20 mL of methanol and 20 mL of 0.025 N sulfuric acid, and shake vigorously for 15 minutes. Transfer the solution to a 50-mL volumetric flask, rinse the centrifuge tube with two 5-mL portions of methanol, and add the rinsings to the flask. Dilute with methanol to volume, and mix.

Assay preparation—Centrifuge a portion of the *Assay stock preparation* for 5 minutes. Immediately prior to injection, transfer 5 mL of the supernatant to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and filter to obtain a solution having a nominal concentration of about 10 µg of meclocycline per mL.

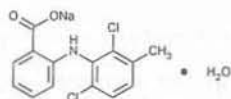
Chromatographic system—The liquid chromatograph is equipped with a 340-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation of the meclocycline peak for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the meclocycline peak. Calculate the percent label claim of C₂₂H₂₁ClN₂O₈ in the portion of Cream taken by the formula:

$$(C_s / C_u)(r_u / r_s)(100)$$

in which C_s is the concentration, in µg per mL, of meclocycline in the *Standard preparation*; C_u is the nominal concentration, in µg per mL, of meclocycline in the *Assay preparation*; and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Meclofenamate Sodium



C₁₄H₁₀Cl₂NNaO₂ · H₂O 336.15

Benzoic acid, 2-[(2,6-dichloro-3-methylphenyl)amino]-, monosodium salt, monohydrate.

Monosodium *N*-(2,6-dichloro-*m*-tolyl)anthranilate monohydrate [6385-02-0].

Anhydrous 318.13

» Meclofenamate Sodium contains not less than 97.0 percent and not more than 103.0 percent of C₁₄H₁₀Cl₂NNaO₂, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—
USP Meclofenamate Sodium RS

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption 1 (197U)—

Solution: 25 µg per mL.

Medium: 0.01 N hydrochloric acid in methanol.

Absorptivities at 242 nm, 279 nm, and 336 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

C: Ultraviolet Absorption 2 (197U)—

Solution: 1 in 40,000.

Medium: 0.1 N sodium hydroxide.

Absorptivities at 279 nm and 317 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

Water Determination, Method I (921): between 4.8% and 5.8%.

Copper—

Standard copper solution—Dissolve 1000 mg of copper wire in 6 mL of nitric acid in a 1 L volumetric flask. Add 8 mL of hydrochloric acid, dilute with water to volume, and mix. Dilute this solution quantitatively and stepwise with water to obtain a *Standard copper solution* having a known concentration of 0.6 µg per mL.

Test solution—Transfer 2 g of Meclofenamate Sodium, accurately weighed, to a 100-mL volumetric flask, and add 1 drop of ammonium hydroxide. Dissolve in water, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard copper solution* and the *Test solution* at the copper emission line at about 325 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a copper hollow-cathode lamp, using water as the blank. Adjust the operating conditions to obtain about 70% full-scale detector response with the *Standard copper solution*. The detector response obtained with the *Test solution* is not greater than that obtained with the *Standard copper solution* (0.003%).

Chromatographic purity—

Standard solutions—Dissolve an accurately weighed quantity of USP Meclofenamate Sodium RS in methanol to obtain a solution containing 20 mg per mL (*Standard solution A*). Dilute 1.0 mL of *Standard solution A* with sufficient methanol to obtain 200 mL of solution (*Standard solution B*).

Test solution—Dissolve 200 mg of Meclofenamate Sodium in 10.0 mL of methanol.

Procedure—Apply 10-µL portions of *Standard solution A*, *Standard solution B*, and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of methylene chloride, methyl ethyl ketone, and glacial acetic acid (50:48:2) until the solvent front has moved about eight-tenths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wave-length UV light: the chromatograms show a principal spot at about the same R_f value, and any secondary spot, if present in the chromatogram from the *Test solution* is not more intense than the principal spot obtained from *Standard solution B* (0.5%).

Assay—Transfer about 350 mg of Meclofenamate Sodium, accurately weighed, to a 125-mL separator, add 10 mL of water, and mix to dissolve. To this solution add 3 mL of 3 N hydrochloric acid, shake, and extract with three 30-mL portions of chloroform, collecting the chloroform extracts in an evaporating flask. Evaporate the chloroform extracts to dry-

ness. Dissolve the residue in 5 mL of dimethyl sulfoxide and 25 mL of methanol. Mix, add 5 drops of phenolphthalein TS, and titrate the mixture with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 31.81 mg of $C_{14}H_{10}Cl_2NNaO_2$.

Meclofenamate Sodium Capsules

» Meclofenamate Sodium Capsules contain an amount of $C_{14}H_{10}Cl_2NNaO_2$ equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of meclofenamic acid ($C_{14}H_{11}Cl_2NO_2$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Meclofenamate Sodium RS

Identification—Prepare a solution of Capsule contents in methanol containing 20 mg per mL, and filter. The clear filtrate so obtained meets the requirements of the *Thin-Layer Chromatographic Identification Test* (201), the solvent mixture consisting of methylene chloride, methyl ethyl ketone, and glacial acetic acid (50:48:2).

Dissolution (711)—

Medium: 0.05 M pH 7.5 phosphate buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Procedure—Determine the amount of meclofenamic acid ($C_{14}H_{11}Cl_2NO_2$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 279 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Meclofenamate Sodium RS in the same *Medium*.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{14}H_{11}Cl_2NO_2$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

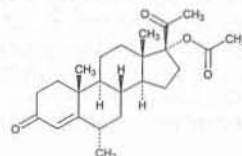
Assay—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to about 50 mg of meclofenamic acid, to a 200-mL volumetric flask. Add 0.01 N hydrochloric acid in methanol to volume, and mix. Filter, discarding the first 20 mL of the filtrate. Transfer 10.0 mL of the filtrate to a 100-mL volumetric flask, add 0.01 N hydrochloric acid in methanol to volume, and mix. Dissolve an accurately weighed quantity of USP Meclofenamate Sodium RS in 0.01 N hydrochloric acid in methanol to obtain a solution having a known concentration of about 27 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 336 nm, with a suitable spectrophotometer, using 0.01 N hydrochloric acid in methanol as the blank. Calculate the quantity, in mg, of meclofenamate acid ($C_{14}H_{11}Cl_2NO_2$) in the portion of Capsule contents taken by the formula:

$$2C(296.15 / 318.13)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Meclofenamate Sodium RS in the Standard solution; 296.15 and 318.13 are the molecular weights of meclofenamic acid and meclofenamate sodium, respectively; and A_U and A_S are

the absorbances of the solution from the Capsule contents and the Standard solution, respectively.

Medroxyprogesterone Acetate



$C_{24}H_{34}O_4$ 386.52
Pregn-4-ene-3,20-dione, 17-(acetyloxy)-6-methyl-, (6α)-;
17-Hydroxy-6α-methylpregn-4-ene-3,20-dione acetate
[71-58-9].

DEFINITION

Medroxyprogesterone Acetate contains NLT 97.0% and NMT 103.0% of medroxyprogesterone acetate ($C_{24}H_{34}O_4$), calculated on the dried basis.

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

• B. ULTRAVIOLET ABSORPTION (197U)

Analytical wavelength: 241 nm

Sample solution: 10 µg/mL in alcohol

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 2.0%.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile and water (40:60)

Standard solution: 1 mg/mL of USP Medroxyprogesterone Acetate RS in acetonitrile

Sample solution: 1 mg/mL of Medroxyprogesterone Acetate in acetonitrile

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) in the portion of Medroxyprogesterone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Medroxyprogesterone Acetate RS in the Standard solution (mg/mL)

C_U = concentration of Medroxyprogesterone Acetate in the Sample solution (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase: Acetonitrile and water (60:40)

System suitability solution: 40 µg/mL each of megesterol acetate and USP Medroxyprogesterone Acetate RS in Mobile phase

Standard solution: 50 µg/mL of USP Medroxyprogesterone Acetate RS in *Mobile phase*

Sample solution: 2.5 mg/mL of Medroxyprogesterone Acetate in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between megesterol acetate and medroxyprogesterone acetate, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Medroxyprogesterone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response of medroxyprogesterone acetate from the *Standard solution*

C_S = concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Medroxyprogesterone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurity: NMT 1.0%

Total impurities: NMT 1.5%

• **LIMIT OF MEDROXYPROGESTERONE ACETATE RELATED COMPOUND A**

Standard solution: 20 mg/mL of USP Medroxyprogesterone Acetate RS and 0.1 mg/mL of USP Medroxyprogesterone Acetate Related Compound A RS in methylene chloride

Sample solution: 20 mg/mL of Medroxyprogesterone Acetate in methylene chloride

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Hexanes, *tert*-butyl methyl ether, and tetrahydrofuran (45:45:10)

Spray reagent: 200 mg/mL of *p*-toluenesulfonic acid in alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about 10 cm. Allow the plate to air-dry, and develop the chromatogram again until the solvent front has moved about 10 cm. Allow the plate to dry at 120° for 10 min. Spray the plate with *Spray reagent*. Heat the plate for 10 min at 120°, and examine the plate under UV light at 365 nm.

Acceptance criteria: NMT 0.5%; any blue fluorescent spot with an R_f value higher than that of the principal spot due to medroxyprogesterone acetate of the *Sample solution* is not more intense than the corresponding blue fluorescent spot of the *Standard solution*.

SPECIFIC TESTS

• **OPTICAL ROTATION, Specific Rotation (781S)**

Sample solution: 10 mg/mL in dioxane

Acceptance criteria: +45° to +51°

• **LOSS ON DRYING (731)**

Analysis: Dry a sample at 105° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

• **USP REFERENCE STANDARDS (11)**

USP Medroxyprogesterone Acetate RS

USP Medroxyprogesterone Acetate Related Compound A RS

4,5β-Dihydromedroxyprogesterone acetate.

C₂₄H₃₆O₄ 388.54

Medroxyprogesterone Acetate Injectable Suspension

DEFINITION

Medroxyprogesterone Acetate Injectable Suspension is a sterile suspension of Medroxyprogesterone Acetate in a suitable aqueous medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of medroxyprogesterone acetate (C₂₄H₃₄O₄).

IDENTIFICATION

• **A. INFRARED ABSORPTION (197K)**

Sample: Transfer a volume of Injectable Suspension, equivalent to 50 mg of medroxyprogesterone acetate, to a centrifuge tube, centrifuge, decant the supernatant, and wash the solids with two 15-mL portions of water, discarding the water washings. Dissolve the solids in 10 mL of chloroform, transfer to a small beaker, evaporate the chloroform on a steam bath, and dry the residue at 105° for 3 h.

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Mobile phase: 700 mL of butyl chloride, 300 mL of hexane, both previously saturated with water, and 80 mL of acetonitrile. The acetonitrile concentration may be varied to meet *System suitability* requirements and to provide elution times of about 12 and 15 min for progesterone and medroxyprogesterone acetate, respectively. Pass the solution through a membrane filter of 1 µm or less pore size.

Internal standard solution: 0.25 mg/mL of progesterone in *Mobile phase*

Standard solution: 0.4 mg/mL of USP Medroxyprogesterone Acetate RS in *Internal standard solution*

Sample solution: Nominally 0.4 mg/mL of medroxyprogesterone acetate in *Internal standard solution*, prepared as follows. Transfer a volume of Injectable Suspension, equivalent to 50 mg of medroxyprogesterone acetate, to a suitable container. Transfer 25 mL of chloroform into the container, shake for 20 min, and centrifuge. Transfer 4 mL of the chloroform layer into a suitable container, and evaporate to dryness. Dissolve the residue in 20 mL of *Internal standard solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 2-mm × 25-cm; 5-µm packing L3

Flow rate: The *Mobile phase* is maintained at a flow rate capable of giving the required resolution and suitable elution times.

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 5.0 between progesterone and medroxyprogesterone acetate

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) in the portion of Injectable Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of medroxyprogesterone acetate to the internal standard from the *Sample solution*

R_S = peak area ratio of medroxyprogesterone acetate to the internal standard from the *Standard solution*

C_S = concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** (791): 3.0–7.0

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS** (11)

USP Medroxyprogesterone Acetate RS

Medroxyprogesterone Acetate Tablets

DEFINITION

Medroxyprogesterone Acetate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$).

IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

Sample: Triturate a number of Tablets, equivalent to about 25 mg of medroxyprogesterone acetate, with 15 mL of chloroform. Filter, evaporate the chloroform on a steam bath, and dry the residue at 105° for 3 h.

Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and water (40:60)

Standard solution: 1 mg/mL of USP Medroxyprogesterone Acetate RS in acetonitrile

Sample solution: Finely powder NLT 20 Tablets. Weigh a portion of the powder, equivalent to 25 mg of medroxyprogesterone acetate, into a 50-mL glass centrifuge tube. Transfer 25 mL of acetonitrile into the tube, shake to wet the powder thoroughly, sonicate for NLT 10 min, and centrifuge. Use the clear supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm \times 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of medroxyprogesterone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• **DISSOLUTION** (711)

Medium: 0.5% sodium lauryl sulfate; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Mobile phase: Acetonitrile and water (60:40)

Sodium lauryl sulfate stock solution: Transfer 180.0 g of sodium lauryl sulfate to a 2000-mL volumetric flask. Add 1500 mL of water, and stir until dissolved. [NOTE—Several hours of stirring are required.] Dilute with water to volume.

Standard stock solution: 70 mg of USP Medroxyprogesterone Acetate RS in 140 mL of *Sodium lauryl sulfate stock solution*. Dilute with water to 250 mL. [NOTE—It may be necessary to sonicate the solution to bring the Reference Standard into solution before dilution with water.] Prepare the *Standard stock solution* fresh daily.

Standard solution: Transfer a 20-mL aliquot of *Standard stock solution* into a 1-L volumetric flask. Add 40 mL of *Sodium lauryl sulfate stock solution*, and dilute with water to volume. This solution is stable for up to 7 days.

Sample solution: Withdraw 15 mL of the solution under test and filter, discarding the first 5 mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm \times 8-cm; packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) dissolved using the responses from the *Sample solution* and *Standard solution*.

Tolerances: NLT 50% (Q) of the labeled amount of medroxyprogesterone acetate ($C_{24}H_{34}O_4$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905)

Procedure for content uniformity

Diluent: Alcohol and water (3:1)

Standard solution: 15 µg/mL of USP Medroxyprogesterone Acetate RS in *Diluent*

Sample solution: Nominally 15 µg/mL of medroxyprogesterone acetate in *Diluent* prepared as follows. Transfer 1 Tablet to a volumetric flask, dilute with *Diluent* to volume, and shake for 15 min. Filter, and quantitatively dilute a portion of the filtrate as needed.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: Maximum at about 242 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of medroxyprogesterone acetate (C₂₄H₃₄O₄) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Medroxyprogesterone Acetate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of medroxyprogesterone acetate in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

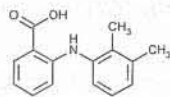
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** (11)

USP Medroxyprogesterone Acetate RS

Mefenamic Acid



C₁₅H₁₅NO₂ 241.29

Benzoic acid, 2-(2,3-dimethylphenyl)amino-

N-2,3-Xylylanthranilic acid [61-68-7].

» Mefenamic Acid contains not less than 98.0 percent and not more than 102.0 percent of C₁₅H₁₅NO₂, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Mefenamic Acid RS

Identification—

A: *Infrared Absorption* (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Loss on drying (731)—Dry it at 105° for 4 hours: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals, Method II** (231): 0.002%. (Official 1-Jan-2018)

Chromatographic purity—

Buffer solution, *Mobile phase*, and *Chromatographic system*—Proceed as directed in the *Assay*.

Standard solution—Dissolve an accurately weighed quantity of USP Mefenamic Acid RS in *Mobile phase* to obtain a solution having a known concentration of about 10 µg per mL.

Test solution—Transfer about 100 mg of Mefenamic Acid, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of each impurity in the portion of Mefenamic Acid taken by the formula:

$$100(C_S / C_U)(r_i / r_S)$$

in which C_S is the concentration, in µg per mL, of USP Mefenamic Acid RS in the *Standard solution*; C_U is the concentration, in µg per mL, of Mefenamic Acid in the *Test solution*; r_i is the peak response for each impurity obtained from the *Test solution*; and r_S is the peak response for mefenamic acid obtained from the *Standard solution*: not more than 0.1% of any individual impurity is found; and not more than 0.5% of total impurities is found.

Assay—

Buffer solution—Prepare a 50 mM solution of monobasic ammonium phosphate, and adjust with 3 M ammonium hydroxide to a pH of 5.0.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, *Buffer solution*, and tetrahydrofuran (23:20:7). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Mefenamic Acid RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer about 100 mg of Mefenamic Acid, accurately weighed, to a 500-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 8200 theoretical plates; the tailing factor for the analyte peak is not more than 1.6; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C₁₅H₁₅NO₂ in the portion of Mefenamic Acid taken by the formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Mefenamic Acid RS in the *Standard preparation*; and r_U and r_S are the mefenamic acid peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Mefenamic Acid Capsules

» Mefenamic Acid Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mefenamic acid ($C_{15}H_{15}NO_2$).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Mefenamic Acid RS

Identification—

A: Place a portion of Capsule contents, equivalent to about 250 mg of mefenamic acid, in a 250-mL volumetric flask, add about 100 mL of a mixture of chloroform and methanol (3:1), and shake vigorously. Dilute with a mixture of chloroform and methanol (3:1) to volume, mix, and filter: the filtrate so obtained responds to the *Thin-layer Chromatographic Identification Test* (201), a solvent system consisting of a mixture of chloroform, ethyl acetate, and glacial acetic acid (75:25:1) and the *Ordinary Impurities* (466) visualization technique 17 being used.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, obtained as directed in the *Assay*.

Dissolution (711)—

0.05 M Tris buffer—Dissolve 60.5 g of tris(hydroxymethyl)aminomethane in 6 L of water, and dilute with water to 10 L. Adjust with phosphoric acid to a pH of 9.0 ± 0.05 . To a second container, transfer about 6 liters of this solution, add 100 g of sodium lauryl sulfate, and mix to dissolve the solid material. Transfer this solution back into the first container, and mix.

Medium: 0.05 M Tris buffer; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{15}H_{15}NO_2$ dissolved, employing the procedure set forth in the *Assay*, making any necessary volumetric adjustments.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{15}H_{15}NO_2$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay* under *Mefenamic Acid*.

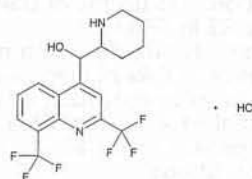
Assay preparation—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Weigh the contents, and determine the average weight per capsule. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to about 100 mg of mefenamic acid, to a 500-mL volumetric flask. Add 10.0 mL of tetrahydrofuran, and sonicate for about 5 minutes with occasional mixing. Dilute with *Mobile phase* to volume, mix, and filter.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Mefenamic Acid*. Calculate the quantity, in mg, of $C_{15}H_{15}NO_2$ in the portion of Capsules taken by the formula:

$$500C(r_u / r_s)$$

in which the terms are as defined therein.

Mefloquine Hydrochloride



$C_{17}H_{16}F_6N_2O \cdot HCl$ 414.77

4-Quinolinemethanol, α -2-piperidinyl-2,8-bis(trifluoromethyl)-, monohydrochloride, (R^*, S^*)- (\pm);

DL-erythro- α -2-Piperidyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol monohydrochloride [51773-92-3].

DEFINITION

Mefloquine Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{17}H_{16}F_6N_2O \cdot HCl$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191)

ASSAY

• PROCEDURE

Solution A: 1.5 g/L of sodium hydrogen sulfate in water

Mobile phase: Dissolve 1 g of tetraheptylammonium bromide in a 1000-mL mixture of acetonitrile, methanol, and *Solution A* (2:1:2).

System suitability solution: 4 μ g/mL each of USP Mefloquine Hydrochloride RS and USP Mefloquine Related Compound A RS in *Mobile phase*

Standard solution: 0.2 mg/mL of USP Mefloquine Hydrochloride RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Mefloquine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Guard column: 4-mm \times 3-cm; C18 (recommended)

Column: 4.0-mm \times 25-cm; 5- μ m packing L1

Column temperature: 25°

Flow rate: 0.8 mL/min

Injection size: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for mefloquine related compound A and mefloquine are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between mefloquine related compound A and mefloquine, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$) in the portion of Mefloquine Hydrochloride taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times 100$$

r_u = peak response of mefloquine from the *Sample solution*

r_s = peak response of mefloquine from the *Standard solution*

C_s = concentration of USP Mefloquine Hydrochloride RS in the *Standard solution* (mg/mL)

C_u = concentration of Mefloquine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-Jan-2018)

ORGANIC IMPURITIES

Mobile phase: Dissolve 1 g of tetraheptylammonium bromide in a 1-L mixture of a 1.5-g/L solution of sodium hydrogen sulfate, acetonitrile, and methanol (2:2:1).

System suitability solution: 4 µg/mL each of USP Mefloquine Hydrochloride RS and USP Mefloquine Related Compound A RS in *Mobile phase*. [NOTE—Mefloquine related compound A is *threo*-mefloquine.]

Sample stock solution: 4 mg/mL of Mefloquine Hydrochloride in *Mobile phase*

Sample solution: 4 µg/mL from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Guard column: 4-mm × 2.5-cm; 5-µm packing L1

Column: 4.0-mm × 25-cm; 5-µm packing L1

Flow rate: 0.8 mL/min

Injection size: 20 µL. [NOTE—Equilibrate the column with *Mobile phase* at a flow rate of 0.8 mL/min for 30 min.]

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for mefloquine related compound A and mefloquine are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between mefloquine related compound A and mefloquine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Sample stock solution* and *Sample solution*
Record the chromatogram for a time that is 10 times the retention time of the main peak.

Acceptance criteria: The response of the mefloquine related compound A peak in the *Sample stock solution* is NMT twice the area of the main peak of the *Sample solution* (0.2%). The response of any other individual peak, other than the main peak of the *Sample stock solution*, is NMT that of the main peak of the *Sample solution* (0.1%); and the sum of the responses of any such peaks of the *Sample stock solution* is NMT five times the response of the main peak of the *Sample solution* (0.5%). [NOTE—Exclude the main peak and any other peak producing a response of less than 0.2 times (0.02%) the main peak of the *Sample solution*.]

SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation** (781S)

Sample solution: 50 mg/mL in methanol

Acceptance criteria: -0.2° to $+0.2^\circ$

- **WATER DETERMINATION, Method I** (921): NMT 3.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 15° and 30° .

USP REFERENCE STANDARDS (11)

USP Mefloquine Hydrochloride RS

USP Mefloquine Related Compound A RS
threo-Mefloquine.

$C_{17}H_{16}F_6N_2O \cdot HCl$ 414.78

Mefloquine Hydrochloride Tablets

DEFINITION

Mefloquine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. ULTRAVIOLET ABSORPTION** (197U)
Diluent, Standard solution, and Sample solution: Proceed as directed in the *Assay*.
Blank: *Diluent*

ASSAY

PROCEDURE

Buffer: 2.7 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0 ± 0.1 .

Diluent: Methanol and water (23:27)

Mobile phase: Methanol, acetonitrile, and *Buffer* (13:10:27)

Standard solution: 0.05 mg/mL of USP Mefloquine Hydrochloride RS in *Diluent*

Sensitivity solution: 0.025 µg/mL of USP Mefloquine Hydrochloride RS in *Diluent*

Sample stock solution: Transfer a suitable number of Tablets to a volumetric flask, dilute with methanol (approximately 80% of the total volume), shake for 30 min, allow to sit for 1 h, and dilute with methanol to volume to obtain a solution having a concentration of 2.5 mg/mL of mefloquine hydrochloride.

Sample solution: Nominally 0.05 mg/mL of mefloquine hydrochloride in *Diluent* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 222 nm

Column: 4.6-mm × 15-cm; 5-µm packing L68

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Column efficiency: NLT 4000 theoretical plates, *Standard solution*

Tailing factor: NMT 1.5, *Standard solution*

Signal-to-noise ratio: NLT 5, *Sensitivity solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Mefloquine Hydrochloride RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of mefloquine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard stock solution: 0.2 mg/mL of USP Mefloquine Hydrochloride RS in Medium. A small amount of methanol, not exceeding 5% of the final volume, may be used to help solubilize mefloquine.

Standard solution: 0.04 mg/mL of USP Mefloquine Hydrochloride RS in Medium from the Standard stock solution

Sample solution: Dilute a portion of the solution under test with Medium (1:5), and pass a portion through a suitable filter of 0.8-μm pore size.

Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

Mode: UV

Analytical wavelength: 285 nm

Cell length: 1 cm

Blank: Medium

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

A_U = absorbance from the Sample solution

A_S = absorbance from the Standard solution

C_S = concentration of USP Mefloquine Hydrochloride RS in the Standard solution (mg/mL)

L = label claim (mg/Tablet)

D = dilution factor of the Sample solution

V = volume of Medium, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: 0.278 mg/mL of USP Mefloquine Hydrochloride RS in Medium. A small amount of methanol, not exceeding 2.5% of the final volume, may be used to help solubilize mefloquine.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

Mode: UV

Analytical wavelength: 284 nm

Cell length: 0.2 cm

Blank: Medium

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance from the Sample solution

A_S = absorbance from the Standard solution

C_S = concentration of USP Mefloquine Hydrochloride RS in the Standard solution (mg/mL)

L = label claim (mg/Tablet)

V = volume of Medium, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of mefloquine hydrochloride ($C_{17}H_{16}F_6N_2O \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Buffer, Diluent, Mobile phase, Standard solution, Sensitivity solution, Sample stock solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the Sample solution

r_S = peak response of mefloquine hydrochloride from the Standard solution

C_S = concentration of USP Mefloquine Hydrochloride RS in the Standard solution (mg/mL)

C_U = nominal concentration of mefloquine hydrochloride in the Sample solution (mg/mL)

Acceptance criteria: See Table 1.

[NOTE—Do not include the threo isomer, a process impurity monitored in the drug substance, in the calculation of total impurities. Disregard any peak less than 0.05%.]

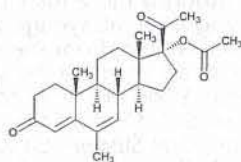
Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Specified (unidentified)	0.67	0.15
Specified (unidentified)	0.70	0.15
threo-Mefloquine (DL-threo-α-2-piperidyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol)	0.75	—
Specified (unidentified)	0.84	0.25
Mefloquine hydrochloride	1.0	—
Any other unknown individual impurity	—	0.15
Total impurities	—	0.50

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one Dissolution test is given, the labeling states the Dissolution test used only if Test 1 is not used.
- **USP REFERENCE STANDARDS (11)**
USP Mefloquine Hydrochloride RS

Megestrol Acetate



$C_{24}H_{32}O_4$ 384.51
Pregna-4,6-diene-3,20-dione, 17-(acetyloxy)-6-methyl-;
17-Hydroxy-6-methylpregna-4,6-diene-3,20-dione acetate
[595-33-5].

DEFINITION

Megestrol Acetate contains NLT 97.0% and NMT 103.0% of megestrol acetate ($C_{24}H_{32}O_4$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile and water (55:45)

Diluent: Acetonitrile and water (40:60)

Internal standard solution: 0.8 mg/mL of propylparaben in acetonitrile

Standard stock solution: 1 mg/mL of USP Megestrol Acetate RS in acetonitrile

Standard solution: 80 µg/mL each of USP Megestrol Acetate RS and propylparaben in *Diluent* from the *Standard stock solution* and *Internal standard solution*, respectively

Sample stock solution: 1 mg/mL of Megestrol Acetate in acetonitrile

Sample solution: 80 µg/mL each of Megestrol Acetate and propylparaben in *Diluent* from the *Sample stock solution* and *Internal standard solution*, respectively

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for propylparaben and megestrol acetate are about 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8.0 between propylparaben and megestrol acetate

Relative standard deviation: NMT 2.0% for the peak response ratio of megestrol acetate to propylparaben

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of megestrol acetate ($C_{24}H_{32}O_4$) in the portion of Megestrol Acetate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of megestrol acetate to propylparaben from the *Sample solution*

R_S = peak response ratio of megestrol acetate to propylparaben from the *Standard solution*
 C_S = concentration of USP Megestrol Acetate RS in the *Standard solution* (mg/mL)
 C_U = concentration of Megestrol Acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%, with a platinum dish being used and ignition at $600 \pm 25^\circ$

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-Jan-2018)

SPECIFIC TESTS

- **COMPLETENESS OF SOLUTION** (641)

Sample: 500 mg in 10 mL of acetone

Acceptance criteria: Meets the requirements

- **MELTING RANGE OR TEMPERATURE** (741): 213° – 220° , but the range between the beginning and the end of melting does not exceed 3° .

- **OPTICAL ROTATION, Specific Rotation** (781S)

Sample solution: 20 mg/mL in chloroform

Acceptance criteria: $+8.8^\circ$ to $+12.0^\circ$ ($t = 20^\circ$)

- **WATER DETERMINATION, Method I** (921): NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.

- **USP REFERENCE STANDARDS** (11)

USP Megestrol Acetate RS

Megestrol Acetate Oral Suspension

DEFINITION

Megestrol Acetate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of megestrol acetate ($C_{24}H_{32}O_4$).

IDENTIFICATION

- **THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

Standard solution: 4.0 mg/mL of USP Megestrol Acetate RS in chloroform

Sample solution: Transfer Oral Suspension, equivalent to 160 mg of megestrol acetate, to a separatory funnel, add 50 mL of water and 40 mL of chloroform, and shake. Allow the phases to separate, and discard the aqueous layer.

Developing solvent system: Chloroform and ethyl acetate (4:1)

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile and water (11:9)

Standard solution: 80 µg/mL of USP Megestrol Acetate RS in *Mobile phase*

Sample solution: A volume of Oral Suspension diluted with *Mobile phase* to obtain nominally 80 µg/mL of megestrol acetate

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection size: 25 µL

System suitabilitySample: *Standard solution***Suitability requirements**

Column efficiency: NLT 2500 theoretical plates

Tailing factor: NMT 1.4

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of $C_{24}H_{32}O_4$ in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Megestrol Acetate RS in the *Standard solution* (µg/mL) C_U = nominal concentration of the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS**• DISSOLUTION (711)****Test 1**

Medium: 0.5% sodium lauryl sulfate in water; 900 mL

Apparatus 2: 25 rpm

Time: 30 min

Detector: UV 292 nm

Standard solution: 45 mg of USP Megestrol Acetate RS in a 250-mL volumetric flask. Add 12 mL of methanol, and place the flask in a warm water bath until the solid is dissolved. Dilute with *Medium* to volume. The final concentration is 180 µg/mL of megestrol acetate. Dilute with *Medium*, if necessary.

Sample solution: Transfer to the surface of the *Medium* in the dissolution vessel an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, equivalent to 160 mg of megestrol acetate. At the sampling time, withdraw a volume of the solution under test and pass through a suitable filter with 0.45-µm pore size. Dilute with *Medium*, if necessary.

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of $C_{24}H_{32}O_4$ released:

$$\text{Result} = (A_U/A_S) \times (C_S/V) \times V_D \times (100/L)$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of the *Standard solution* (mg/mL) V = volume of Oral Suspension taken V_D = volume of *Medium*, 900 mL L = label claim (mg/mL)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{24}H_{32}O_4$ is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.5% sodium lauryl sulfate in water; 900 mL

Apparatus 2: 25 rpm

Time: 30 min

Detector: UV 292 nm, using 0.5-cm pathlength cuvettes

Standard solution: 45 mg of USP Megestrol Acetate RS in a 250-mL volumetric flask. Add 5 mL of methanol. Dilute with *Medium* to volume. Transfer 10 mL of this solution to a 100-mL volumetric flask, and dilute

with *Medium* to volume. The final concentration is 18 µg/mL.

Sample solution: [NOTE—Use a separate syringe for each vessel.] Withdraw more than 10 mL of the Oral Suspension, using a 10-mL syringe with a long cannula. Remove air bubbles from the syringe. Adjust the volume to the 10-mL mark on the syringe, and remove the needle. Wipe the tip of the syringe, and weigh (gross weight). Operate the apparatus, and rapidly dispense the Oral Suspension to the side of the vessel at about halfway from the bottom. Similarly dispense the Oral Suspension into other vessels. Weigh each syringe after dispensing the sample (tare weight). Record sample weights. After completion of the dissolution, pass an aliquot through a suitable nylon filter with 0.45-µm pore size, and dilute 2.0 mL of the filtrate with *Medium* to 50.0 mL to obtain a solution having a theoretical concentration of about 18 µg/mL.

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of $C_{24}H_{32}O_4$ released:

$$\text{Result} = (A_U/A_S) \times (C_S/W) \times V_D \times d \times (100/L)$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of the *Standard solution* (mg/mL) W = weight of the Oral Suspension taken (mg) V_D = volume of the *Medium* in the dissolution vessel, 900 mL d = density of the Oral Suspension (mg/mL), obtained by dividing the weight of Oral Suspension taken by 10 mL L = label claim (mg/mL)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{24}H_{32}O_4$ is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 0.5% sodium lauryl sulfate in degassed water; 900 mL. [NOTE—Use ultrapure sodium lauryl sulfate with an Assay content of NLT 99.0%.]

Apparatus 2: 50 rpm

Time: 30 min

Determine the amount of $C_{24}H_{32}O_4$ dissolved by using the following method.

Mobile phase: Proceed as directed in the Assay.

Standard solution: 0.46 mg/mL of USP Megestrol Acetate RS in *Mobile phase*

Sample solution: Proceed as directed for *Test 2*, introducing the sample into the vessel over a 10- to 15-s period (about 1 mL/s).

Chromatographic system: Proceed as directed in the Assay.

Injection size: 10 µL

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of $C_{24}H_{32}O_4$ released:

$$\text{Result} = (r_U/r_S) \times (C_S/W) \times V_D \times d \times (100/L)$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of the *Standard solution* (mg/mL) W = weight of the Oral Suspension taken (mg) V_D = volume of the *Medium* in the dissolution vessel, 900 mL d = density of the Oral Suspension (mg/mL), obtained by dividing the weight of Oral Suspension taken by 10 mL L = label claim (mg/mL)

Tolerances: NLT 80% (Q) of the labeled amount of $C_{24}H_{32}O_4$ is dissolved.

- **DELIVERABLE VOLUME** (698): Meets the requirements

SPECIFIC TESTS

- **PH** (791): 3.0–4.7

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **LABELING:** When more than one test for *Dissolution* is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)
USP Megestrol Acetate RS

Megestrol Acetate Tablets

DEFINITION

Megestrol Acetate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of megestrol acetate ($C_{24}H_{32}O_4$).

[NOTE—Megestrol Acetate Tablets labeled solely for veterinary use are exempt from the requirements of the *Dissolution* test.]

IDENTIFICATION

- **A.**
Sample solution: Grind a suitable number of Tablets in a known volume of chloroform, NLT 10 mL, to obtain a solution containing 4 mg/mL of megestrol acetate.
Analysis: Filter the *Sample solution* into a beaker. Pipet 0.6 mL of the filtrate into a stainless steel grinding vial containing 500 mg of potassium bromide, dry with a current of air, grind, pellet, and record the IR spectrum.
Acceptance criteria: The IR absorption spectrum of the potassium bromide dispersion so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Megestrol Acetate RS.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile and water (55:45)
Diluent: Acetonitrile and water (40:60)
Internal standard solution: 0.8 mg/mL of propylparaben in acetonitrile
Standard stock solution: 1 mg/mL of USP Megestrol Acetate RS in acetonitrile
Standard solution: 80 µg/mL each of USP Megestrol Acetate RS and propylparaben in *Diluent* from the *Standard stock solution* and *Internal standard solution*, respectively
Sample solution: Nominally 80 µg/mL of megestrol acetate prepared as follows. Transfer the equivalent of 80 mg of megestrol acetate from powdered Tablets (NLT 20 Tablets) to a 100-mL volumetric flask. Add 10 mL of water, and shake for 10 min. Add 75 mL of acetonitrile, shake for 30 min, then dilute with acetonitrile to volume. Place a 25-mL aliquot in a glass-stoppered 35-mL centrifuge tube, insert the stopper, and centrifuge for 10 min. Transfer 5.0 mL of the supernatant and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for propylparaben and megestrol acetate are about 0.4 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8.0 between propylparaben and megestrol acetate

Relative standard deviation: NMT 2.0% for the peak response ratio of megestrol acetate to propylparaben

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of megestrol acetate ($C_{24}H_{32}O_4$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of megestrol acetate to propylparaben from the *Sample solution*

R_S = peak response ratio of megestrol acetate to propylparaben from the *Standard solution*

C_S = concentration of USP Megestrol Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of megestrol acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• DISINTEGRATION (701)

Sample: Tablets labeled solely for veterinary use; proceed as directed for plain-coated Tablets, but use film-coated Tablets instead.

Time: 30 min

Acceptance criteria: Meet the requirements

• DISSOLUTION (711)

Medium: 1% sodium lauryl sulfate; 900 mL

Apparatus 2: 75 rpm

Time: 60 min

Standard solution: USP Megestrol Acetate RS in *Medium*

Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

Instrumental conditions

Analytical wavelength: UV 292 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the amount of megestrol acetate ($C_{24}H_{32}O_4$) dissolved.

Tolerances: NLT 75% (Q) of the labeled amount of megestrol acetate ($C_{24}H_{32}O_4$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Standard solution: 10 µg/mL of USP Megestrol Acetate RS in methanol

Sample solution: Nominally 10 µg/mL of megestrol acetate prepared as follows. Place 1 Tablet in a volumetric flask of suitable size so that the final expected solution concentration is between 0.2 and 1.0 mg of megestrol acetate per mL. Add 1 mL of water, and gently shake until the Tablet has disintegrated. Fill the flask to three-quarters of its nominal capacity with methanol, and shake by mechanical means for 20 min. Dilute with methanol to volume, mix, and filter, discarding the first 15 mL of the filtrate. Dilute 5.0 mL of the subsequent filtrate with methanol.

Instrumental conditions

Mode: UV-Vis

Wavelength range: 260–350 nm

Analytical wavelength: Absorption maximum at about 288 nm

Cell: 1 cm

Blank: Methanol

Analysis

Samples: Standard solution, Sample solution, and Blank

Record the absorbances of the Standard solution and the Sample solution against the Blank, scanning from 260 to 350 nm.

Calculate the percentage of megestrol acetate ($C_{24}H_{32}O_4$) in the Tablet taken:

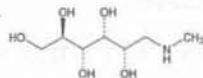
$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

 A_U = absorbance of the Sample solution A_S = absorbance of the Standard solution C_S = concentration of USP Megestrol Acetate RS in the Standard solution ($\mu\text{g/mL}$) C_U = nominal concentration of megestrol acetate in the Sample solution ($\mu\text{g/mL}$)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Tablets intended solely for veterinary use are so labeled.
- **USP REFERENCE STANDARDS (11)**
USP Megestrol Acetate RS

Meglumine $C_7H_{17}NO_5$ D-Glucitol, 1-deoxy-1-(methylamino)-;
1-Deoxy-1-(methylamino)-D-glucitol [6284-40-8].

195.21

DEFINITIONMeglumine contains NLT 99.0% and NMT 100.5% of meglumine ($C_7H_{17}NO_5$), calculated on the dried basis.**IDENTIFICATION****A.**

Sample solution: Transfer 250 mg to a dry, 50-mL centrifuge tube, add 500 mg of sodium metaperiodate, then add 5 mL of water rapidly in one portion. Allow to stand undisturbed.

Analysis: The solution instantly turns yellow, and heat is produced. The color then changes from deep yellow to orange-brown (rust), and after 20 min, the rust-colored solution is cloudy. Then add 2 mL of 2.5 N sodium hydroxide.

Acceptance criteria: The mixture turns bright yellow and becomes clear.

ASSAY**PROCEDURE**

Sample: 500 mg

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N hydrochloric acid

Endpoint detection: Visual

Analysis: Transfer the Sample into a conical flask, dissolve in 40 mL of water, and add 2 drops of methyl red

TS. Titrate with Titrant. Each mL of Titrant is equivalent to 19.52 mg of meglumine ($C_7H_{17}NO_5$).

Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES**Delete the following:**• **HEAVY METALS (231)**

Test preparation: Dissolve 1 g in 20 mL of water, add phenolphthalein TS, neutralize with 3 N hydrochloric acid, and dilute with water to 25 mL.

Acceptance criteria: NMT 20 $\mu\text{g/g}$ (Official 1-Jan-2018)• **RESIDUE ON IGNITION (281):** NMT 0.1%**SPECIFIC TESTS**• **ABSENCE OF REDUCING SUBSTANCES**

Sample solution: 50 mg/mL

Analysis: To 5 mL of Sample solution add 5 mL of alkaline cupric tartrate TS, and heat to boiling.

Acceptance criteria: The color of the solution does not change.

• **COMPLETENESS AND COLOR OF SOLUTION**

Sample solution: 200 mg/mL

Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

Mode: Vis

Analytical wavelength: 420 nm

Cell: 1 cm

Blank: Water

Analysis

Sample: Sample solution

Acceptance criteria: Absorbance is NMT 0.030.

• **LOSS ON DRYING (731)**

Sample: 1 g

Analysis: Dry the Sample at 105° to constant weight.

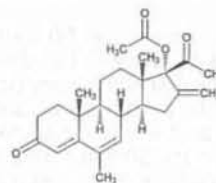
Acceptance criteria: NMT 1.0%

• **MELTING RANGE OR TEMPERATURE (741):** 128°–132°• **OPTICAL ROTATION, Specific Rotation (7815)**

Sample solution: 100 mg/mL, undried, in water

Acceptance criteria: -15.7° to -17.3° **ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Melengestrol Acetate $C_{25}H_{32}O_4$ 396.52

Pregna-4,6-diene-3,20-dione, 17-(acetyloxy)-6-methyl-16-methylene-

17-Hydroxy-6-methyl-16-methylenepregna-4,6-diene-3,20-dione acetate [2919-66-6].

» Melengestrol Acetate contains not less than 97.0 percent and not more than 103.0 percent of $C_{25}H_{32}O_4$, calculated on the dried basis.**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.**Labeling**—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Melengestrol Acetate RS

USP Melengestrol Acetate Related Compound A RS
16-Methylene-17 α -hydroxy-4-pregnene-3,20-dione
17-acetate.USP Melengestrol Acetate Related Compound B RS
17 α -Hydroxy-6,16-dimethyleneprogna-4-ene-3,20-dione
17-acetate.**Identification**—**A:** *Infrared Absorption* (197K).**B:** *Ultraviolet Absorption* (197U)—*Solution:* 10 μ g per mL.*Medium:* alcohol.**C:** The retention time of the melengestrol acetate peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.**Melting temperature** (741): between 219° and 226°.**Specific rotation** (781S): between -132.0° and -122.0°, at 20°.*Test solution:* 10.0 mg per mL, in chloroform.**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.**Delete the following:**• **Heavy metals**, *Method II* (231): not more than 0.001%.

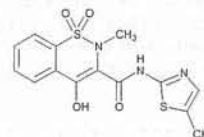
• (Official 1-Jan-2018)

Related compounds—*Mobile phase*—Prepare a mixture of acetonitrile and water (50:50).*Standard solution*—Dissolve an accurately weighed quantity of USP Melengestrol Acetate RS, USP Melengestrol Acetate Related Compound A RS, and USP Melengestrol Acetate Related Compound B RS in methanol to obtain a solution having known concentrations of about 0.005 mg of each per mL.*Test solution*—Use the *Assay preparation*.*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a multiwavelength detector set at 240 and 262 nm and a 4.6-mm \times 25-cm column that contains 5- μ m packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure* [NOTE—Melengestrol acetate and melengestrol related compound B will generate larger peak areas at 262 nm than at 240 nm; melengestrol acetate related compound A will generate a larger peak area at 240 nm than at 262 nm]: the relative retention times are about 0.78, 1.0, and 1.05 for melengestrol acetate related compound A, melengestrol acetate, and melengestrol acetate related compound B, respectively; the resolution, R , between melengestrol acetate related compound A and melengestrol acetate related compound B is not less than 5.0; the column efficiency for the melengestrol acetate related compound A peak is greater than 1500 theoretical plates; the tailing factor is less than 2.0; and the relative standard deviation for replicate injections is not more than 5.0%.*Procedure*—Separately inject equal volumes (about 20 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, identify the peaks, and determine which detector wavelength generates the larger peak area for each impurity. Using the larger peak area, calculate the percentage of each impurity in the portion of Melengestrol Acetate taken by the formula:

$$100(C_s / C_u)(r_i / r_s)$$

in which C_s is the concentration, in mg per mL, of either melengestrol related compound A or melengestrol relatedcompound B in the *Standard solution* [NOTE—If using the impurity peak area generated at 240 nm, C_s is the concentration of melengestrol related compound A; if using the impurity peak area generated at 262 nm, C_s is the concentration of melengestrol related compound B]; C_u is the concentration, in mg per mL, of melengestrol acetate in the *Test solution*; r_i is the peak area of each impurity obtained from the *Test solution*; and r_s is the peak area of either melengestrol related compound A or melengestrol related compound B obtained from the *Standard solution* [NOTE—If using the impurity peak area generated at 240 nm, r_s is the peak area of melengestrol related compound A; if using the impurity peak area generated at 262 nm, r_s is the peak area of melengestrol related compound B]; not more than 0.5% of any unidentified impurity is found; not more than 0.2% of any unidentified impurity is found; and not more than 1.0% of total impurities is found.**Assay**—*Mobile phase*—Prepare a mixture of acetonitrile and water (50:50).*Standard preparation*—Dissolve an accurately weighed quantity of USP Melengestrol Acetate RS in methanol to obtain a solution having a known concentration of about 0.5 mg per mL.*Assay preparation*—Transfer about 50 mg of Melengestrol Acetate, accurately weighed, to a 100-mL volumetric flask, and dissolve in and dilute with methanol to volume.*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 287-nm detector and a 4.6-mm \times 25-cm column that contains 5- μ m packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation* as directed for *Procedure*: the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2.0%.*Procedure*—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* in duplicate into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{25}H_{32}O_4$ in the portion of Melengestrol Acetate taken by the formula:

$$2CW(r_u / r_s)$$

in which C is the concentration, in mg per mL, of the *Standard preparation*; W is the weight, in mg, of Melengestrol Acetate used to prepare the *Assay preparation*; r_u is the average peak area of the melengestrol acetate peak obtained from the *Assay preparation*; and r_s is the average peak area of the melengestrol acetate peak obtained from the *Standard preparation*.**Meloxicam** $C_{14}H_{13}N_3O_4S_2$ 351.40
4-Hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide [71125-38-7].**DEFINITION**Meloxicam contains NLT 98.0% and NMT 102.0% of meloxicam ($C_{14}H_{13}N_3O_4S_2$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the meloxicam peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY• **PROCEDURE**

Solution A: Mixture of a 0.1% (w/v) solution of ammonium acetate adjusted with 10% ammonia solution to a pH of 9.1

Mobile phase: Methanol and *Solution A* (21:29)

Diluent: Methanol and 1 N sodium hydroxide (250:1)

System suitability solution: 0.08 mg/mL each of USP Meloxicam RS and USP Meloxicam Related Compound A RS prepared as follows. Dissolve USP Meloxicam RS and USP Meloxicam Related Compound A RS in 50% of the flask volume of *Diluent*, and dilute with water to volume.

Standard solution: 0.2 mg/mL of USP Meloxicam RS prepared as follows. Dissolve USP Meloxicam RS in 50% of the flask volume of *Diluent*, and dilute with water to volume.

Sample solution: 0.2 mg/mL of Meloxicam prepared as follows. Dissolve Meloxicam in 50% of the flask volume of *Diluent*, and dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 360 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for meloxicam related compound A and meloxicam are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between meloxicam related compound A and meloxicam, *System suitability solution*

Tailing factor: NMT 2.0 for the meloxicam peak, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of meloxicam ($C_{14}H_{13}N_3O_4S_2$) in the portion of Meloxicam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of meloxicam from the *Sample solution*

r_S = peak response of meloxicam from the *Standard solution*

C_S = concentration of USP Meloxicam RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

Solution A: 0.1% (w/v) solution of monobasic potassium phosphate adjusted with 1 N sodium hydroxide to a pH of 6.0

Solution B: Methanol

Diluent: Methanol and 1 N sodium hydroxide (50:3)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
2	60	40
10	30	70
15	30	70
15.1	60	40
18	60	40

System suitability solution: 0.08 mg/mL each of USP Meloxicam RS, USP Meloxicam Related Compound A RS, and USP Meloxicam Related Compound B RS prepared as follows. Dissolve USP Meloxicam RS, USP Meloxicam Related Compound A RS, and USP Meloxicam Related Compound B RS in 10% of the flask volume of *Diluent*, and dilute with water to volume.

Standard stock solution: 0.6 mg/mL of USP Meloxicam RS prepared as follows. Dissolve USP Meloxicam RS in 25% of the flask volume of *Diluent*, and dilute with methanol to volume.

Standard solution: 0.012 mg/mL of USP Meloxicam RS in methanol from the *Standard stock solution*

Sample solution: 4 mg/mL of Meloxicam prepared as follows. Dissolve Meloxicam in 25% of the flask volume of *Diluent*, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 and 350 nm (variable wavelength or multi-wavelength detector)

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 5 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are listed in *Table 2*.]

Suitability requirements

Resolution: NLT 3.0 between meloxicam related compound A and meloxicam at 350 nm; NLT 3.0 between meloxicam related compound B and meloxicam at 260 nm, *System suitability solution*

Relative standard deviation: NMT 10%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Meloxicam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of meloxicam at 350 nm from the *Standard solution*

C_S = concentration of USP Meloxicam RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1-Jan-2018)

• **ORGANIC IMPURITIES, PROCEDURE 1**

Perform either *Procedure 1* or *Procedure 2*, depending on the manufacturing process used.

Table 2

Name	Relative Retention Time	Wavelength (nm)	Relative Response Factor	Acceptance Criteria, NMT (%)
Meloxicam related compound B ^a	0.4	260	1.0	0.1
Meloxicam	1.0	—	—	—
Meloxicam related compound A ^b	1.4	350	0.5	0.1
Methyl-meloxicam ^c	1.7	350	1.0	0.05
Ethyl-meloxicam ^d	1.9	350	1.0	0.05
Individual unknown impurity	—	260/350	1.0	0.1
Total impurities	—	—	—	0.3

^a 5-Methylthiazol-2-amine.

^b Ethyl 4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxylate 1,1-dioxide.

^c N-[3,5-Dimethylthiazol-2(3H)-ylidene]-4-hydroxy-2-methyl-2H-benzo[e][1,2]thiazine-3-carboxamide 1,1-dioxide.

^d N-[3-Ethyl-5-methylthiazol-2(3H)-ylidene]-4-hydroxy-2-methyl-2H-benzo[e][1,2]thiazine-3-carboxamide 1,1-dioxide.

[NOTE—For the specified impurities, calculate the percentage content of each impurity, using the peak responses from the *Sample solution* recorded at the detection wavelength given in Table 2. For an unknown impurity, calculate the percentage content, using peak responses recorded at the wavelength that gives the greater response.]

Acceptance criteria: See Table 2.

• ORGANIC IMPURITIES, PROCEDURE 2

If an article complies with this test, the labeling indicates that it meets the requirements under *Organic Impurities, Procedure 2*.

Solution A and Solution B: Proceed as directed in *Procedure 1*.

Mobile phase: See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	45	55
25	45	55
30	30	70
40	30	70
45	45	55
50	45	55

Diluent A: Diluent B and 0.4 N sodium hydroxide (50:3)

Diluent B: Methanol and water (2:3)

Standard stock solution A: 0.01 mg/mL of USP Meloxicam RS prepared as follows. Dilute a solution of 0.05 mg/mL of USP Meloxicam RS in Diluent A with Diluent B.

Standard stock solution B: 0.05 mg/mL each of USP Meloxicam Related Compound B RS and USP Meloxicam Related Compound C RS prepared as follows. Transfer suitable amounts of USP Meloxicam Related Compound B RS and USP Meloxicam Related Compound C RS to an adequate volumetric flask. Add 0.4 N sodium hydroxide to 6% of the flask volume, and sonicate for 2 min. Add an additional 40% of the flask volume of methanol, sonicate for 2 min, and dilute with water to volume.

Standard solution: 0.001 mg/mL of USP Meloxicam RS and 0.0015 mg/mL each of USP Meloxicam Related Compound B RS and USP Meloxicam Related Compound C RS prepared as follows. Transfer suitable volumes of *Standard stock solution A* and *Standard stock solution B* to an adequate volumetric flask, and dilute with Diluent B to volume.

Sample solution: 1 mg/mL of Meloxicam prepared as follows. Dissolve a suitable amount of Meloxicam with 50% of the flask volume of Diluent A, and dilute with Diluent B to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV variable wavelength or multi-wavelength detector at 260 and 350 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times are listed in Table 4.]

Suitability requirements

Relative standard deviation: NMT 5.0% for meloxicam, meloxicam related compound B, and meloxicam related compound C

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Meloxicam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of the corresponding related compound from the *Standard solution*

C_S = concentration of the corresponding USP Related Compound RS in the *Standard solution* (mg/mL). [NOTE—Use the concentration of USP Meloxicam RS for unknown impurities.]

C_U = concentration of the *Sample solution* (mg/mL)

[NOTE—Use the peak response and concentration of USP Meloxicam RS for unknown impurities; for the specified impurities, calculate the percentage content of each impurity using the *Sample solution* peak responses recorded at the detection wavelength given in Table 4. For an unknown impurity, calculate the percentage content using peak responses recorded at the wavelength that gives the greater response.]

Acceptance criteria: See Table 4.

Table 4

Name	Relative Retention Time	Wavelength (nm)	Acceptance Criteria, NMT (%)
Meloxicam	1.0	350	—
Meloxicam related compound B ^a	0.8	260	0.1

^a 5-Methylthiazol-2-amine.

^b Isopropyl-4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxylate-1,1-dioxide.

Table 4 (Continued)

Name	Relative Retention Time	Wave-length (nm)	Acceptance Criteria, NMT (%)
Meloxicam related compound C ^b	3.2	350	0.1
Individual unknown impurity	—	260/350	0.1
Total impurities	—	—	0.3

^a 5-Methylthiazol-2-amine.^b Isopropyl-4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxylate-1,1-dioxide.**SPECIFIC TESTS****• LOSS ON DRYING (731)**

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS**• PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.**• LABELING:** The labeling states with which *Procedure* under *Organic Impurities* the article complies if a test other than *Procedure 1* is used.**• USP REFERENCE STANDARDS (11)**

USP Meloxicam RS

USP Meloxicam Related Compound A RS

Ethyl 4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxylate 1,1-dioxide.

C₁₂H₁₃NO₅S 283.30

USP Meloxicam Related Compound B RS

5-Methylthiazol-2-amine.

C₄H₆N₂S 114.175

USP Meloxicam Related Compound C RS

Isopropyl-4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxylate-1,1-dioxide.

C₁₃H₁₅NO₅S 297.33**Meloxicam Oral Suspension**

» Meloxicam Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of meloxicam (C₁₄H₁₃N₃O₄S₂).

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards (11)—

USP Meloxicam RS

USP Meloxicam Related Compound B RS

2-Amino-5-methyl-thiazole.

Identification—**A: Thin-Layer Chromatographic Identification Test (201)**—

Test solution—Transfer a volume of Oral Suspension, equivalent to about 2.5 mg of meloxicam, to a 10-mL volumetric flask. Dilute with acetone to volume, and mix for 10 minutes. If necessary, pass through fluted filter paper.

Standard solution: 0.25 mg per mL, prepared by dissolving USP Meloxicam RS in 1 mL of water and diluting with acetone to volume.

Developing solvent solution: a mixture of chloroform, methanol, and ammonium hydroxide (80:20:1)

Procedure—Proceed as directed in the chapter. After removing the plate from the chamber and drying, examine the chromatograms under UV light at 254-nm: the *R_f* value (approximately 0.45) of the principal dark spot obtained

from the *Test solution* corresponds to that obtained from the *Standard solution*.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

pH (791): between 3.5 and 4.5.

Viscosity—Rotational Methods (912)—Determine using a shear rate programmable rotational viscometer: between 40 and 100 centipoises, determined at 20°.

Dissolution (711)—**Medium:** pH 7.5 phosphate buffer; 900 mL.**Apparatus 2:** 25 rpm.**Time:** 15 minutes.

Determine the amount of C₁₄H₁₃N₃O₄S₂ dissolved by employing the following method.

Standard solution—Transfer about 20.83 mg of USP Meloxicam RS, accurately weighed, into a 100-mL volumetric flask. Dissolve in 5 mL of methanol and 1 mL of 0.1 M sodium hydroxide, and dilute with *Medium* to volume. Dilute with *Medium* to a final concentration of about 8.3 µg per mL of meloxicam.

Test solution—Shake each sample for 15 minutes. Weigh six portions, equivalent to 7.5 mg of the Oral Suspension, into separate tared 10-mL beakers, and record each weight. Introduce each of the samples to the middle of the dissolution vessels, and rinse each beaker with about 20 mL of the *Medium* withdrawn from the vessel. Carefully lower the paddle to the appropriate height and start the rotation. After completion of the dissolution, pass a 20-mL aliquot through a nylon filter having a 0.45-µm porosity, discarding the first 3 mL of the filtrate.

Procedure—Determine the amount of C₁₄H₁₃N₃O₄S₂ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 362 nm on the *Test solution* in comparison with the *Standard solution*, using *Medium* as the blank. Calculate the percentage of C₁₄H₁₃N₃O₄S₂ released by the formula:

$$\frac{A_U \times C_S \times 900 \times d \times 100}{A_S \times W_U \times LC}$$

in which *A_U* and *A_S* are the absorbances obtained from the *Test solution* and the *Standard solution*, respectively; *C_S* is the concentration, in mg per mL, of the *Standard solution*; *d* is the density, in g per mL, of the Oral Suspension; *W_U* is the weight, in mg, of the Oral Suspension taken; 900 is the volume, in mL, of the *Medium*; 100 is the conversion factor to percentage; and *LC* is the label claim, in mg per mL.

Tolerances—Not less than 75% (*Q*) of the labeled amount of C₁₄H₁₃N₃O₄S₂ is dissolved in 15 minutes.

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—The total aerobic microbial count does not exceed 100 cfu per g or 100 cfu per mL. The total yeasts and molds count does not exceed 50 cfu per g or 50 cfu per mL. It meets the requirements of the test for the absence of *Escherichia coli*.

Chromatographic purity—

Buffer, Mobile phase, and Diluent—Proceed as directed in the *Assay*.

Related compound standard stock solution—Proceed as directed for *Related compound standard stock preparation* in the *Assay*.

Sensitivity solution—Dilute the *Related compound standard stock solution* with *Diluent* to a final concentration of about 0.08 µg per mL.

Related compound standard solution—Dilute *Related compound standard stock preparation* with *Diluent* to a final concentration of about 0.5 µg per mL.

Test solution—Proceed as directed for Assay preparation in the Assay.

Chromatographic system (see Chromatography (621))—Proceed as directed in the Assay. Chromatograph the *Sensitivity solution* (about 10 μ L), and record the peak responses as directed for Procedure at 260 nm: the relative standard deviation of three replicate injections is not more than 10% for meloxicam related compound B. Chromatograph the *Related compound standard solution* (about 10 μ L), and record the peak responses as directed for Procedure at 260 nm: the tailing factor for meloxicam related compound B is not more than 2.0.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Related compound standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and record the peak areas at 260 nm and 360 nm. The run time is about 20 minutes or two times the retention time of meloxicam. Calculate the percentage of meloxicam related compound B in the portion of Oral Suspension taken by the formula:

$$(5000/L)(C/V)(r_U / r_S)$$

in which L is the label claim, in mg per mL; C is the concentration, in mg per mL, of USP Meloxicam Related Compound B RS in the *Related compound standard solution*; V is the volume, in mL, of Oral Suspension taken to prepare the *Test solution*; r_U is the peak area obtained for meloxicam related compound B in the *Test solution* at 260 nm; and r_S is the peak area for meloxicam related compound B in the *Related compound standard solution* at 260 nm. Calculate the percentage of each unknown degradation product in the portion of Oral Suspension taken by the formula:

$$100(r_i / r_S)$$

in which r_i is the area of any unknown degradant at 360 nm; r_S is the sum of areas of meloxicam and all impurities in the *Test solution* at 360 nm. Not more than 0.15% of meloxicam related compound B is found; not more than 0.2% of any individual unknown degradation product is found; and not more than 0.5% of total degradation products is found.

Assay—

Buffer—Dissolve 2 g of monohydrate citric acid and 2 g of boric acid in 1000 mL of water, and adjust with dihydrate trisodium citrate to a pH of 2.9.

Mobile phase—Mix 565 mL of Buffer, 260 mL of methanol, and 200 mL of acetonitrile. Degas the solution, and then dissolve 200 mg of sodium dodecyl sulfate in 1000 mL of the resulting solution.

Diluent—Dissolve 3 g of boric acid and 1.5 g of dihydrate trisodium citrate in 1000 mL of water, and adjust with 2 M sodium hydroxide to a pH of 8.3. Mix 420 mL of the resulting buffer with 420 mL of methanol and 160 mL of acetonitrile.

Standard stock preparation—Transfer about 67 mg of USP Meloxicam RS, accurately weighed, into a 100-mL volumetric flask. Add 3.0 mL of dimethylformamide. Swirl the flask, and allow to stand for about 5 minutes. Add 15 mL of methanol. Dilute with Diluent to just below volume. Sonicate for 30 minutes, and mix until dissolved. Cool to room temperature. Dilute with Diluent to volume.

Standard preparation—Dilute Standard stock preparation with Diluent to a final concentration of about 0.27 mg per mL.

Related compound standard stock preparation—Transfer about 21 mg of USP Meloxicam Related Compound B RS, accurately weighed, into a 100-mL volumetric flask. Add 3.0 mL of dimethylformamide, 15 mL of methanol, and about 60 mL of Diluent. Sonicate, and mix until dissolved. Cool to room temperature. Dilute with Diluent to volume.

Dilute further with Diluent to a concentration of about 8.4 μ g per mL.

System suitability solution—Transfer a volume of Oral Suspension, equivalent to about 15 mg of meloxicam, accurately weighed, to a 50-mL volumetric flask. Add 3.0 mL of *Related compound standard stock preparation*. Add 3.0 mL of dimethylformamide. Swirl the flask, and allow to stand for about 5 minutes. Add 15 mL of methanol. Dilute with Diluent to just below volume. Sonicate for 30 minutes, mixing the flask vigorously about every 5 minutes. Cool to room temperature. Dilute with Diluent to volume. Mix, and allow particulates to settle. Pass through a 0.45- μ m membrane filter with a fiberglass prefilter.

Assay preparation—Transfer an accurately measured volume of Oral Suspension, equivalent to about 15 mg of meloxicam, to a 50-mL volumetric flask. Add 3.0 mL of dimethylformamide. Swirl the flask, and allow to stand for about 5 minutes. Add 15 mL of methanol. Dilute with Diluent to just below volume. Sonicate for 30 minutes, mixing the flask vigorously about every 5 minutes. Cool to room temperature. Dilute with Diluent to volume. Mix, and allow particulates to settle. Pass through a 0.45- μ m membrane filter with a fiberglass prefilter.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a programmable dual wavelength detector, a single wavelength detector in series, or a photodiode array detector capable of detecting wavelengths from 190 nm to 400 nm, or equivalent, and a 4-mm \times 12.5-cm analytical column that contains 5- μ m packing L1. The column temperature is maintained at 40°. The flow rate is about 1.0 mL per minute. The run time is about 20 minutes or two times the retention time of meloxicam. Chromatograph the *System suitability solution* (about 10 μ L), and record the peak responses as directed for Procedure at 360 nm and 260 nm: at 360 nm the resolution, R , between meloxicam and any other adjacent peak is not less than 1.5. The tailing factor for the meloxicam peak is not more than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for Procedure at 360 nm: the relative standard deviation for replicate injections of the *Standard preparation* is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and record the peak areas at 360 nm. Calculate the amount of meloxicam ($C_{14}H_{13}N_3O_4S_2$), in mg per mL, in the portion of Oral Suspension taken by the formula:

$$50(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Meloxicam RS in the *Standard preparation*; V is the volume, in mL, of Oral Suspension taken to prepare the *Assay preparation*; r_U is the peak area obtained for meloxicam in the *Assay preparation* at 360 nm; and r_S is the peak area for meloxicam in the *Standard solution* at 360 nm.

Meloxicam Tablets

» Meloxicam Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of meloxicam ($C_{14}H_{13}N_3O_4S_2$).

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards (11)—
USP Meloxicam RS

Identification—

A: Thin-Layer Chromatographic Identification Test (201)—

0.1 N Methanolic sodium hydroxide—Dilute 100 mL of 1 N sodium hydroxide with methanol to 1000 mL.

Test solution—Transfer a portion of finely powdered Tablets, equivalent to about 50 mg of meloxicam, to a suitable flask. Add 5 mL of 0.1 N Methanolic sodium hydroxide, and mix. Add 20 mL of methanol, and stir for about 15 minutes. Filter the mixture to remove insoluble material, and use the filtrate.

Standard solution—Transfer about 20 mg of USP Meloxicam RS, accurately weighed, to a 10-mL volumetric flask, dissolve in 2 mL of 0.1 N Methanolic sodium hydroxide, dilute with methanol to volume, and mix.

Developing solvent system—Prepare a mixture of chloroform, methanol, and ammonia water (25%) (80:20:1).

Procedure—Proceed as directed in the chapter.

B: The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

Dissolution (711)—

Medium: pH 7.5 phosphate buffer (prepared by dissolving 6.81 g of potassium dihydrogen phosphate in 800 mL of water, adjusting the pH to 7.5 with 0.5 N sodium hydroxide, and diluting with water to 1 L); 900 mL.

Apparatus 2: 75 rpm.

Time: 30 minutes.

Determine the amount of meloxicam dissolved by employing the following method.

Standard solution—

FOR TABLETS LABELED TO CONTAIN 7.5 MG—Transfer about 33.3 mg of USP Meloxicam RS, accurately weighed, to a 100-mL volumetric flask. Add 5.0 mL of methanol, 1.0 mL of 0.1 N sodium hydroxide, dilute with Medium to volume, and mix. Transfer 5.0 mL to a 100-mL volumetric flask, dilute with Medium to volume, and mix. Transfer 25.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with Medium to volume, and mix.

FOR TABLETS LABELED TO CONTAIN 15 MG—Transfer about 33.3 mg of USP Meloxicam RS, accurately weighed, to a 100-mL volumetric flask. Add 5.0 mL of methanol, 1.0 mL of 0.1 N sodium hydroxide, dilute with Medium to volume, and mix. Transfer 5.0 mL to a 100-mL volumetric flask, dilute with Medium to volume, and mix.

Test solution—Use portions of the solution under test passed through a suitable 10-μm filter, discarding the first few mL.

Procedure—Determine the percentage of the labeled amount of meloxicam dissolved by employing UV absorption, using a suitable spectrophotometer, at the wavelength of maximum absorbance at about 362 nm, using 1-cm cuvettes, on the Test solution in comparison with the Standard solution using Medium as blank. Calculate the percentage of meloxicam dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times LC}$$

in which A_U and A_S are the absorbances obtained from the Test solution and the Standard solution, respectively; C_S is the concentration, in mg per mL, of the Standard solution; 900 is the volume, in mL, of Medium; 100 is the conversion factor to percentage; and LC is the Tablet label claim, in mg.

Tolerances—Not less than 70% (Q) of the labeled amount of meloxicam is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Related compounds—

Solution A, Solution B, and Mobile phase—Proceed as directed in the Assay.

Standard solution—Use the Standard preparation from the Assay.

System sensitivity solution—Transfer 4 mL of the Standard solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 5 mL of the resulting solution to a 50-mL volumetric flask, add 5 mL of 1 N sodium hydroxide, and dilute with methanol to volume.

Test solution—Use the Assay preparation.

Chromatographic system (see Chromatography (621))—Proceed as directed in the Assay, except to chromatograph the Standard solution and the System sensitivity solution: the tailing factor for the meloxicam peak is not more than 2.0; the relative standard deviation for replicate injections of the Standard solution is not more than 2.0%; and the signal-to-noise ratio of the meloxicam peak in the chromatogram of the System sensitivity solution is not less than 10.

Procedure—Separately inject equal volumes (about 25 μL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the peak responses. Determine the relative retention times for the impurity peaks relative to that of the meloxicam peak. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$(5000/3)(1/F)(C/W)(A/L)(r_i/r_s)$$

in which F is the relative response factor for each impurity and is equal to 2.7 for the impurity with a relative retention time of about 0.5 (meloxicam related compound B [2-amino-5-methylthiazole]) and 1.0 for all other impurities; C is the concentration, in mg per mL, of USP Meloxicam RS in the Standard solution; W is the weight, in mg, of powdered Tablets taken to prepare the Test solution; A is the average weight of a Tablet; L is the labeled amount, in mg, of meloxicam in each Tablet; r_i is the peak response obtained for each impurity in the Test solution; and r_s is the peak response for meloxicam in the Standard solution: not more than 0.15% of meloxicam related compound B is found; not more than 0.2% of any individual unknown impurity is found; and not more than 0.5% of total impurities is found.

Assay—

Solution A—Dissolve 2.0 g of dibasic ammonium phosphate in 1 L of water, and adjust with phosphoric acid to a pH of 7.0 ± 0.1 .

Solution B—Mix 650 mL of methanol and 100 mL of isopropyl alcohol.

Mobile phase—Prepare a filtered and degassed mixture of Solution A and Solution B (63:37). Make adjustments if necessary (see System Suitability under Chromatography (621)).

Standard stock preparation—[NOTE—The Standard stock preparation is prepared so that the final concentration of meloxicam, in mg per mL, is approximately equivalent to the concentration of the Assay stock preparation.] Transfer a suitable quantity of USP Meloxicam RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 1 mL of 1 N sodium hydroxide and 30 mL of methanol, and dilute with methanol to volume. Transfer 10 mL of the resulting solution to a 100-mL volumetric flask, add 10 mL of 1 N sodium hydroxide, and dilute with methanol to volume.

Standard preparation—Transfer 15 mL of the Standard stock preparation to a 25-mL volumetric flask, and dilute with water to volume.

Assay stock preparation—Transfer 10 Tablets to a 1000-mL volumetric flask, add about 100 mL of 1 N sodium hydroxide, shake to disperse the Tablets, and add 800 mL of methanol. Sonicate the solution for about 15 minutes, then stir for 30 minutes. Dilute with methanol to volume, and mix. Filter the resulting solution, and use the filtrate.

Assay preparation—Transfer 15 mL of the *Assay stock preparation* to a 25-mL volumetric flask, and dilute with water to volume.

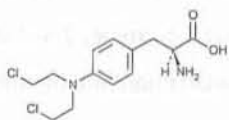
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector, a guard column that contains packing L1, and a 4-mm \times 10-cm column that contains packing L1. The flow rate is about 0.8 mL per minute. The column temperature is maintained at 40°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the meloxicam peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 μ L) of the *Standard preparation* and the *Assay preparation* to the chromatograph, record the chromatograms, and measure the responses for the meloxicam peak. Calculate the quantity, in mg, of meloxicam ($C_{14}H_{13}N_3O_4S_2$) in the portion of Tablets taken by the formula:

$$5000(C/3)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Meloxicam RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Melphalan



$C_{13}H_{18}Cl_2N_2O_2$ 305.20

L-Phenylalanine, 4-bis(2-chloroethyl)amino]-

L-3-[p-[Bis(2-chloroethyl)amino]phenyl]alanine [148-82-3].

» Melphalan contains not less than 93.0 percent and not more than 100.5 percent of $C_{13}H_{18}Cl_2N_2O_2$, calculated on the dried and ionizable chlorine-free basis.

Caution—Handle Melphalan with exceptional care because it is a highly potent agent.

Packaging and storage—Preserve in tight, light-resistant, glass containers.

USP Reference standards (11)—

USP Melphalan Hydrochloride RS

Identification—

A: Ultraviolet Absorption (197U)—

Solution: 5 μ g per mL.

Medium: methanol.

B: To 1 mL of 1 in 10,000 solution in alcohol in a glass-stoppered test tube add 1 mL of pH 4.0 acid phthalate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*), 1 mL of a 1 in 20 solution of 4-(p-nitrobenzyl)pyridine in acetone, and 1 mL of saline TS. Heat on a water bath at 80° for 20 minutes, and cool quickly. Add 10 mL of alcohol and 1 mL of 1 N potassium hydroxide: a violet to red-violet color is produced.

C: Heat 100 mg with 10 mL of 0.1 N sodium hydroxide on a water bath for 10 minutes; the resulting solution, after acidification with 2 N nitric acid, responds to the tests for Chloride (191).

Specific rotation (781S): between -30° and -36° .

Test solution: 7 mg per mL, in methanol, prepared with the aid of gentle heating.

Loss on drying (731): Dry it in vacuum at 105° to constant weight: it loses not more than 7.0% of its weight.

Residue on ignition (281): not more than 0.3%.

Ionizable chlorine—Dissolve about 500 mg of Melphalan, accurately weighed, in a mixture of 75 mL of water and 2 mL of nitric acid, allow to stand for 2 minutes, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically: not more than 1.0 mL of 0.1 N silver nitrate is required for each 500 mg of test specimen.

Nitrogen Determination (461)—Determine the nitrogen content as directed under *Method II*, using about 325 mg of Melphalan, accurately weighed, and 0.1 N sulfuric acid VS for the titration: not less than 8.90% and not more than 9.45% of N is found, calculated on the dried basis.

Assay—Transfer to a beaker about 200 mg of Melphalan, accurately weighed, and dissolve in 20 mL of 0.5 N sodium hydroxide. Cover the beaker with a watch glass, and boil the solution for 30 minutes, adding water as necessary to maintain the volume. Cool, neutralize to phenolphthalein TS with acetic acid, and add 1 mL of acetic acid in excess. Titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using silver and calomel electrodes, the latter modified to contain saturated potassium sulfate solution. From the results obtained in the test for *Ionizable chlorine*, calculate the volume, in mL, of 0.1 N silver nitrate that is equivalent to the ionizable chlorine in the quantity of Melphalan taken for the *Assay*, and subtract it from the *Assay* titration volume. Each mL of 0.1 N silver nitrate is equivalent to 15.26 mg of $C_{13}H_{18}Cl_2N_2O_2$.

Melphalan Tablets

» Melphalan Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of melphalan ($C_{13}H_{18}Cl_2N_2O_2$).

Packaging and storage—Preserve in well-closed, light-resistant, glass containers.

USP Reference standards (11)—

USP Melphalan Hydrochloride RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: Shake a portion of finely powdered Tablets, equivalent to about 2 mg of melphalan, with 20 mL of alcohol, and filter: a 1-mL portion of the solution so obtained responds to *Identification* test B under *Melphalan*.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{13}H_{18}Cl_2N_2O_2$ dissolved by employing the following method.

Mobile phase—Transfer 2 grams of ammonium acetate, 2 mL of glacial acetic acid, and 0.4 mL of triethylamine to a suitable flask containing 1500 mL of water and 500 mL of acetonitrile. Stir until all solids are dissolved and well mixed, then filter and degas.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm \times 5-cm column that contains packing L7.

The flow rate is about 1.5 mL per minute. Chromatograph replicate injections of the Standard solution, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 3.0%.

Procedure—Inject a volume (about 50 μ L) of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the quantity of $C_{13}H_{18}Cl_2N_2O_2$ dissolved in comparison with a Standard solution having a known concentration of USP Melphalan Hydrochloride RS in the same *Medium* and similarly chromatographed.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{13}H_{18}Cl_2N_2O_2$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Procedure for content uniformity—Place 1 Tablet in a 200-mL volumetric flask, add 10 mL of water and 10 mL of alcohol, sonicate to dissolve the soluble components in the mixture, dilute with alcohol to volume, mix, and filter to obtain a clear solution. Dissolve an accurately weighed quantity of USP Melphalan Hydrochloride RS in alcohol to obtain a Standard solution having a known concentration of about 10 μ g per mL. Concomitantly determine the absorbances of the solution from the Tablet and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 260 nm, with a suitable spectrophotometer, using alcohol as the blank. Calculate the quantity, in mg, of melphalan ($C_{13}H_{18}Cl_2N_2O_2$) in the Tablet taken by the formula:

$$(305.20/341.66)(T/D)C(A_U / A_S)$$

in which 305.20 and 341.66 are the molecular weights of melphalan and melphalan hydrochloride, respectively; *T* is the labeled quantity, in mg, of melphalan in the Tablet; *D* is the concentration, in μ g per mL, of melphalan in the solution from the Tablet, on the basis of the labeled quantity per Tablet and the extent of dilution; *C* is the concentration, in μ g per mL, of USP Melphalan Hydrochloride RS in the Standard solution; and *A_U* and *A_S* are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—

Mobile phase—Prepare a solution of 0.025 M diethylamine in a mixture of methanol and water (1:1), adjust with 3.5 N hydrochloric acid to a pH of 5.5, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Melphalan Hydrochloride RS in alcohol, and quantitatively dilute with alcohol to obtain a solution having a known concentration of about 0.9 mg of melphalan hydrochloride per mL. Pipet 10 mL of this solution into a 100-mL volumetric flask containing 75 mL of alcohol and 2.0 mL of glacial acetic acid, dilute with alcohol to volume, and mix to obtain a *Standard preparation* having a known concentration of about 90 μ g of USP Melphalan Hydrochloride RS per mL (equivalent to about 80 μ g of melphalan per mL).

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 8 mg of anhydrous melphalan, to a 100-mL volumetric flask. Add about 75 mL of alcohol and 2.0 mL of glacial acetic acid to the flask, and sonicate for 15 minutes. Cool, dilute with alcohol to volume, and mix. Filter through a medium-porosity, sintered-glass funnel, discarding the first few mL of the filtrate, and use the remainder of the filtrate as the *Assay preparation*.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector

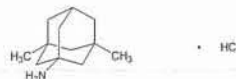
and a 4.2-mm \times 25-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (between 10 and 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of melphalan ($C_{13}H_{18}Cl_2N_2O_2$) in the portion of Tablets taken by the formula:

$$(305.20/341.67)(0.1C)(r_U / r_S)$$

in which 305.20 and 341.67 are the molecular weights of melphalan and melphalan hydrochloride, respectively; *C* is the concentration, in μ g per mL, of melphalan hydrochloride in the *Standard preparation*; and *r_U* and *r_S* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Memantine Hydrochloride



$C_{12}H_{21}N \cdot HCl$
Tricyclo[3.3.1.1^{3,7}]decan-1-amine, 3,5-dimethyl-, hydrochloride;
1-Amino-3,5-dimethyladamantane hydrochloride
[41100-52-1].

215.76

DEFINITION

Memantine Hydrochloride contains NLT 98.0% and NMT 102.0% of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements

ASSAY

• PROCEDURE

Internal standard solution: 4.0 mg/mL of adamantane in *n*-hexane

Standard solution: 4.0 mg/mL of USP Memantine Hydrochloride RS in *Internal standard solution* prepared as follows. Transfer 100 mg of USP Memantine Hydrochloride RS to a 50-mL centrifuge tube. Add 15 mL of 1 N sodium hydroxide, and mix. Add 25 mL of *Internal standard solution*, and shake for 15 min. Allow the layers to separate, and filter a portion of the top hexane layer through anhydrous sodium sulfate. Use the clear filtrate.

Sample solution: 4.0 mg/mL of Memantine Hydrochloride in *Internal standard solution* prepared as follows. Transfer 100 mg of Memantine Hydrochloride to a 50-mL centrifuge tube. Add 15 mL of 1 N sodium hydroxide, and mix. Add 25 mL of *Internal standard solution*, and shake for 15 min. Allow the layers to separate, and filter a portion of the top hexane layer through anhydrous sodium sulfate. Use the clear filtrate.

Chromatographic system(See *Chromatography* <621>, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 50-m × 0.32-mm; 0.52-μm packing G27

Temperatures

Injection port: 220°

Detector: 300°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	5	145	0
145	10	250	20

Carrier gas: Helium

Flow rate: 4.0 ± 0.4 mL/min

Injection volume: 1 μL

Injection type: Split ratio, 1:50

System suitabilitySample: *Standard solution***Suitability requirements**

Tailing factor: NMT 2.0 each for memantine and adamantane

Relative standard deviation: NMT 2.0% for the ratio of the peak areas of adamantane and memantine

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of memantine hydrochloride (C₁₂H₂₁N · HCl) in the portion of Memantine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of memantine to the internal standard from the *Sample solution*

R_S = peak response ratio of memantine to the internal standard from the *Standard solution*

C_S = concentration of USP Memantine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Memantine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES**Delete the following:**

• **HEAVY METALS**, *Method II* <231>: NMT 10 ppm • (Official 1-

Jan-2018)

• **RESIDUE ON IGNITION** <281>: NMT 0.1%

• **ORGANIC IMPURITIES**

Standard stock solution A: 2.5 mg/mL each of USP Memantine Related Compound A RS, USP Memantine Related Compound B RS, USP Memantine Related Compound C RS, USP Memantine Related Compound D RS, and USP Memantine Related Compound E RS in *n*-hexane

Standard stock solution B: 2.5 mg/mL of USP Memantine Hydrochloride RS prepared as follows. To the flask containing a weighed amount of USP Memantine Hydrochloride RS, add 5.0 N sodium hydroxide to fill 20% of the final volume and *n*-hexane to fill 20% of the final volume. Shake for 10 min, and transfer the contents to a separator. Allow the layers to separate, filter a portion of the top hexane layer, dry the organic layer by swirling with anhydrous sodium sulfate, and allow to

stand for a few min to ensure all the remaining water has been removed. Use the clear filtrate.

System suitability solution: 25 μg/mL each of USP Memantine Related Compound A RS, USP Memantine Related Compound B RS, USP Memantine Related Compound C RS, USP Memantine Related Compound D RS, and USP Memantine Related Compound E RS, from *Standard stock solution A* in *Standard stock solution B*. The concentration of USP Memantine Hydrochloride RS is 2.5 mg/mL.

Standard solution: 25 μg/mL each of USP Memantine Related Compound A RS, USP Memantine Related Compound B RS, USP Memantine Related Compound C RS, USP Memantine Related Compound D RS, USP Memantine Related Compound E RS, and USP Memantine Hydrochloride RS, from *Standard stock solution A* and *Standard stock solution B*, respectively, in *n*-hexane

Sample solution: 25 mg/mL of Memantine Hydrochloride prepared as follows. Transfer the weighed amount of Memantine Hydrochloride to a suitable volumetric flask. Add 5.0 N sodium hydroxide to fill 30% of the final volume and *n*-hexane to fill 40% of the final volume. Shake for 10 min, and transfer the contents to a separator. Allow the layers to separate, filter a portion of the top hexane layer, dry the organic layer by swirling with anhydrous sodium sulfate, and allow to stand for a few min to ensure all the remaining water has been removed. Use the clear filtrate.

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for the relative retention times.]

Suitability requirements

Resolution: NLT 6.0 between memantine and memantine related compound B; NLT 2.0 between memantine related compound B and memantine related compound C, *System suitability solution*

Tailing factor: NMT 2.0 for memantine, *Standard solution*

Relative standard deviation: NMT 10.0% for memantine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Ignore the peaks at the relative retention times 0.11, 0.12, 0.13, 0.18, and 0.26 with respect to the memantine peak, as they correspond to residual solvents.]

Calculate the percentage of each of memantine related compounds A, B, C, D, and E in the portion of Memantine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of memantine related compounds A, B, C, D, or E from the *Sample solution*

r_S = peak response of the corresponding USP Memantine Related Compound RS from the *Standard solution*

C_S = concentration of the corresponding USP Memantine Related Compound RS in the *Standard solution* (mg/mL)

C_U = concentration of Memantine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Memantine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other impurity from the *Sample solution*

r_S = peak response of memantine hydrochloride from the *Standard solution*

- C_s = concentration of USP Memantine Hydrochloride RS in the *Standard solution* (mg/mL)
 C_u = concentration of Memantine Hydrochloride in the *Sample solution* (mg/mL)
Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Memantine related compound A	0.77	0.15
Memantine	1.0	—
Memantine related compound B	1.03	0.15
Memantine related compound C	1.07	0.15
Memantine related compound D	1.19	0.15
Memantine related compound E	1.44	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	0.50

SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* (921): NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
 - USP Memantine Hydrochloride RS
 - USP Memantine Related Compound A RS
1,3-Dimethyladamantane.
 $C_{12}H_{20}$ 164.29
 - USP Memantine Related Compound B RS
3,5-Dimethyladamantane-1-ol.
 $C_{12}H_{20}O$ 180.29
 - USP Memantine Related Compound C RS
1-Chloro-3,5-dimethyladamantane.
 $C_{12}H_{19}Cl$ 198.73
 - USP Memantine Related Compound D RS
1-Bromo-3,5-dimethyladamantane.
 $C_{12}H_{19}Br$ 243.18
 - USP Memantine Related Compound E RS
N-3,5-Dimethyladamantan-1-yl formamide.
 $C_{13}H_{21}NO$ 207.31

Memantine Hydrochloride Tablets**DEFINITION**

Memantine Hydrochloride Tablets contain an amount of memantine hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
 Analytical range: 4000–400 cm^{-1}
Standard: 6.7 mg/mL of USP Memantine Hydrochloride RS in dichloromethane. Shake for 10 min, and pass through a suitable filter. Evaporate the solvent at room temperature. Collect the residue powder, and dry at 60° for 15 min. Prepare an approximate 1% (w/w) dispersion of the sample in potassium bromide.

Sample: 6.7 mg/mL of memantine hydrochloride in dichloromethane from NLT 20 crushed Tablets. Shake for 10 min, and centrifuge for 10 min. Pass the supernatant through a suitable filter. Evaporate the solvent at room temperature. Collect the residue powder, and dry at 60° for 15 min. Prepare an approximate 1% (w/w) dispersion of the sample in potassium bromide.

Acceptance criteria: Fingerprint region of the *Standard* and *Sample* spectrum exhibit maxima at the same wave numbers.

- **B.** The retention time of the memantine peak of the *Sample solution* corresponds to that of the memantine peak of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: 200 mg/mL of sodium hydroxide in water

Internal standard solution: 25 $\mu g/mL$ of USP

Amantadine Hydrochloride RS in water

Standard stock solution: 25 $\mu g/mL$ of USP Memantine Hydrochloride RS prepared as follows. Weigh a suitable quantity of the Standard into a volumetric flask. Add methanol to fill 40% of the final flask volume, and sonicate. Dilute with water to volume.

Standard solution: Pipet 4.0 mL each of the *Internal standard solution* and the *Standard stock solution* into a test tube. Add 2 mL of *Solution A*, and mix on a vortex mixer for 1 min. Add 4.0 mL of toluene, and mix on a vortex mixer for 3 min. Allow the two layers to separate. Inject the toluene layer.

Sample stock solution: Nominally 20 $\mu g/mL$ of memantine hydrochloride prepared as follows. Transfer a suitable number of Tablets to a volumetric flask to obtain a 0.1 mg/mL memantine hydrochloride solution. Add methanol to fill 40% of the final flask volume, and sonicate for 30 min with intermittent shaking. Add water to fill 40% of the final flask volume, and sonicate for 30 min with intermittent shaking. Dilute with water to volume, and centrifuge a portion for 10 min. Pipet a suitable volume of the clear centrifugate into a volumetric flask, and dilute with water to volume.

Sample solution: Pipet 5.0 mL of the *Sample stock solution*, 4.0 mL of the *Internal standard solution*, and 2 mL of *Solution A* into a test tube, and mix on a vortex mixer for 1 min. Add 4.0 mL of toluene, and mix on a vortex mixer for 5 min. Allow the two layers to separate. Inject the toluene layer.

Blank: To 5.0 mL of 80 $\mu L/mL$ of methanol in water add 2 mL of *Solution A*, and mix on a vortex mixer for 1 min. Add 4.0 mL of toluene, and mix on a vortex mixer for 5 min. Allow the two layers to separate. Inject the toluene layer.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m \times 0.32-mm; 0.25- μm packing G27

Temperatures

Injection port: 210°

Detector: 300°

Oven: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	2
50	20	140	0
140	30	200	5

Carrier gas: Helium
Flow rate: 34.8 psi
Injection volume: 4 μ L
Injection type: Split ratio, 1:10

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for amantadine and memantine are 0.97 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between amantadine and memantine

Tailing factor: NMT 2.5 for amantadine; NMT 2.0 for memantine

Relative standard deviation: NMT 2.0% for the ratio of the peak areas of amantadine and memantine

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of the labeled amount of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of memantine to amantadine from the *Sample solution*

R_S = peak area ratio of memantine to amantadine from the *Standard solution*

C_S = concentration of USP Memantine Hydrochloride RS in the *Standard solution* (μ g/mL)

C_U = nominal concentration of memantine hydrochloride in the *Sample solution* (μ g/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: 0.1 N hydrochloric acid with sodium chloride (2 g/L of sodium chloride in water), adjusted with hydrochloric acid to a pH of 1.2; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard stock solution: (L/900) mg/mL of USP Memantine Hydrochloride RS in *Medium*, where L is the label claim in mg/Tablet

Internal standard solution: 28 μ g/mL of USP Amantadine Hydrochloride RS in *Medium*

Standard solution

For Tablets labeled to contain 5 mg: Transfer 5 mL of the *Standard stock solution* to a test tube, add 1 mL of the *Internal standard solution* and 2 mL of 5 N sodium hydroxide, and mix for 1 min. Add 3 mL of toluene, and mix for 2 min. Use the toluene layer.

For Tablets labeled to contain 10 mg: Transfer 5 mL of the *Standard stock solution* to a test tube, add 2 mL of the *Internal standard solution* and 2 mL of 5 N sodium hydroxide, and mix for 1 min. Add 3 mL of toluene, and mix for 2 min. Use the toluene layer.

Sample solution: Pass a portion of the solution under test through a suitable filter.

For Tablets labeled to contain 5 mg: Transfer 5 mL of the filtrate to a test tube, add 1 mL of the *Internal standard solution* and 2 mL of 5 N sodium hydroxide, and mix for 1 min. Add 3 mL of toluene, and mix for 2 min. Use the toluene layer.

For Tablets labeled to contain 10 mg: Transfer 5 mL of the filtrate to a test tube, add 2 mL of the *Internal standard solution* and 2 mL of 5 N sodium hydroxide, and mix for 1 min. Add 3 mL of toluene, and mix for 2 min. Use the toluene layer.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC, splitless

Detector: Flame ionization

Column: 30-m \times 0.32-mm; 0.25- μ m packing G27

Flow rate: 34.8 psi

Temperatures

Injection port: 210°

Detector: 300°

Oven: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	2
50	20	140	0
140	30	200	5

Carrier gas: Helium

Injection volume: 4 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between amantadine and memantine

Tailing factor: NMT 2.0 each for amantadine and memantine

Relative standard deviation: NMT 2.0% for the ratio of memantine to amantadine peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$) dissolved:

$$\text{Result} = (R_U/R_S) \times (C_S/L) \times V \times 100$$

R_U = peak area ratio of memantine to amantadine from the *Sample solution*

R_S = peak area ratio of memantine to amantadine from the *Standard solution*

C_S = concentration of USP Memantine Hydrochloride RS in the *Standard solution* (μ g/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES**Change to read:**• **LIMIT OF MEMANTINE-LACTOSE ADDUCT**

[NOTE—Perform this test if lactose is present in the formulation.]

Solution A: 40 mg/mL of sodium hydroxide in water

Buffer: Dissolve 3.3 g of monobasic potassium phosphate and 2.3 g of sodium 1-octane sulfonate in 1 L of water. Adjust with *Solution A* to a pH of 6.1.

Mobile phase: Acetonitrile, methanol, and *Buffer* (26:4:70)

Standard solution: 0.2 mg/mL of USP Memantine Hydrochloride RS in *Mobile phase*

Sample solution: Nominally 10 mg/mL of memantine hydrochloride from NLT 25 crushed Tablets, prepared as follows. Transfer an amount of powder equivalent to 100 mg of memantine hydrochloride to a 20-mL volumetric flask. Add 10 mL of *Mobile phase*, and sonicate

for 30 min. Centrifuge, and pass a portion of the centrifugate through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Temperatures

Column: 40°

Detector: 35°

Flow rate: 1.3 mL/min

Injection volume: 50 μ L

Run time: 1.3 times the retention time of the memantine peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 3.5

Relative standard deviation: NMT 10.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the memantine-lactose adduct in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the memantine-lactose adduct from the *Sample solution*

r_S = peak response of memantine from the *Standard solution*

C_S = concentration of USP Memantine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of memantine hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor of the memantine-lactose adduct (see *Table 3*)

Acceptance criteria: See *Table 3*.

Disregard all peaks other than the memantine-lactose adduct peak.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Memantine-lactose adduct	0.41	0.53	1.4 [●] (RB 1-Apr-2016)
Memantine	1.0	1.0	—

Change to read:

• ORGANIC IMPURITIES

Solution A: 1 N sodium hydroxide

System suitability stock solution A: 0.5 mg/mL each of USP Memantine Related Compound A RS, USP Memantine Related Compound B RS, USP Memantine Related Compound C RS, USP Memantine Related Compound D RS, and USP Memantine Related Compound E RS in *n*-hexane

System suitability stock solution B: Transfer 75 mg of USP Memantine Hydrochloride RS into a suitable container, add 9 mL of 1.0 N sodium hydroxide and 6 mL of *n*-hexane, and mix for 10 min.

System suitability solution: Pipet 4.0 mL of the *n*-hexane layer from *System suitability stock solution B* into a 10-mL volumetric flask. Add 0.5 mL of *System suitability stock solution A*, and dilute with *n*-hexane to volume.

Standard stock solution: 1.3 mg/mL of USP Memantine Hydrochloride RS in *n*-hexane prepared as follows. Weigh a suitable quantity of the Standard into a volumetric flask. Add *Solution A* to fill 30% of the final flask

volume, and mix for 5 min. Add *n*-hexane to fill 40% of the final flask volume, and shake for 10 min. Transfer the contents of the flask into a separator. Allow the layers to separate, and filter a portion of the top *n*-hexane layer through anhydrous sodium sulfate. Use the clear solution.

Standard solution: Pipet 2.0 mL of the clear solution from the *Standard stock solution* into a 100-mL volumetric flask, and dilute with *n*-hexane to volume.

Sample solution: Nominally 5 mg/mL of memantine hydrochloride in *n*-hexane from NLT 20 crushed Tablets, prepared as follows. Transfer a weighed amount of powder equivalent to 100 mg of memantine hydrochloride to a suitable volumetric flask. Add *Solution A* to fill 15% of the final flask volume. Shake to disperse the material, and then shake for 5 min. Sonicate for 5 min with intermittent shaking. Add *n*-hexane to fill 20% of the final flask volume, and shake for 10 min. Transfer the contents into a separator. Allow the layers to separate, and filter a portion of the top hexane layer through anhydrous sodium sulfate. Use the clear solution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 50-m \times 0.32-mm; 0.52- μ m packing G27

Temperatures

Injection port: 220°

Detector: 300°

Oven: See *Table 4*.

Table 4

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	2
50	5	145	0
145	10	250	20

Carrier gas: Helium

Flow rate: 4.0 \pm 0.2 mL/min

Injection volume: 3 μ L

Injection type: Split ratio, 1:20

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 5* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between memantine and memantine related compound B; NLT 2.0 between memantine related compound B and memantine related compound C, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of memantine related compound E or any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of memantine related compound E or any individual degradation product from the *Sample solution* (ERR 1-Apr-2016)

r_S = peak response of memantine hydrochloride from the *Standard solution*

C_S = concentration of USP Memantine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of memantine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 5.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Memantine related compound A ^a	0.77	—
Memantine	1.0	—
Memantine related compound B ^a	1.03	—
Memantine related compound C ^a	1.1	—
Memantine related compound D ^a	1.2	—
Memantine related compound E	1.4	0.3
Any individual unspecified degradation product	—	0.20
Total impurities ^b	—	0.5

^a Process impurities controlled in the drug substance and are included for identification only. Not reported for the drug product and not included in the total impurities.

^b Excludes memantine-lactose adduct monitored in the test for *Limit of Memantine-Lactose Adduct*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

- **USP REFERENCE STANDARDS (11)**

USP Amantadine Hydrochloride RS

USP Memantine Hydrochloride RS

USP Memantine Related Compound A RS

1,3-Dimethyladamantane.

$C_{12}H_{20}$ 164.29

USP Memantine Related Compound B RS

3,5-Dimethyladamantane-1-ol.

$C_{12}H_{20}O$ 180.29

USP Memantine Related Compound C RS

1-Chloro-3,5-dimethyladamantane.

$C_{12}H_{19}Cl$ 198.73

USP Memantine Related Compound D RS

1-Bromo-3,5-dimethyladamantane.

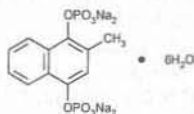
$C_{12}H_{19}Br$ 243.18

USP Memantine Related Compound E RS

N-3,5-Dimethyladamantan-1-yl formamide.

$C_{13}H_{21}NO$ 207.31

Menadiol Sodium Diphosphate



$C_{11}H_8Na_4O_8P_2 \cdot 6H_2O$ 530.17

1,4-Naphthalenediol, 2-methyl-, bis(dihydrogen phosphate), tetrasodium salt, hexahydrate.

2-Methyl-1,4-naphthalenediol bis(dihydrogen phosphate) tetrasodium salt, hexahydrate [6700-42-1].

Anhydrous 422.09 [131-13-5].

» Menadiol Sodium Diphosphate contains not less than 97.5 percent and not more than 102.0 percent of $C_{11}H_8Na_4O_8P_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store in a cold place.

Identification—

A: Dissolve about 200 mg of Menadiol Sodium Diphosphate in 10 mL of water, add 10 mL of 2 N sulfuric acid, 10 mL of 0.1 N ceric sulfate, and 1 mL of 30 percent hydrogen peroxide previously diluted with 5 mL of water, and extract the solution with two 10-mL portions of chloroform. Gently evaporate the clear chloroform solution on a steam bath to dryness, and dry the residue at 80° for 1 hour: the menadione so obtained melts between 104° and 107°.

B: To 50 mg of the dried residue obtained in *Identification* test A add 5 mL of water, then add 75 mg of sodium bisulfite, and heat on a steam bath, shaking vigorously until the substance is dissolved and the solution is practically colorless. Dilute with water to 50 mL, and mix. To 2 mL of the solution add 2 mL of alcoholic ammonia (prepared by mixing equal volumes of alcohol and ammonium hydroxide), shake, and add 3 drops of ethyl cyanoacetate: a deep purplish blue color is produced, and on the addition of 1 mL of sodium hydroxide solution (1 in 3), it changes to green and then to yellow.

C: To about 20 mg contained in a small beaker add 1 mL of water, 2 drops of nitric acid, and 1 mL of sulfuric acid, and heat slowly to the evolution of white fumes. Cool, cautiously dilute with water to about 10 mL, and filter if not clear. Render the filtrate slightly alkaline to litmus with 6 N ammonium hydroxide, then render it acid with nitric acid, and add to the warm solution 3 mL of ammonium molybdate TS: a yellow precipitate is formed within a few minutes.

Water Determination, Method I (921): between 19.0% and 21.5%.

Assay—Dissolve about 100 mg of Menadiol Sodium Diphosphate, accurately weighed, in 25 mL of water, and add 25 mL of glacial acetic acid and 25 mL of 3 N hydrochloric acid. Titrate the solution with 0.02 N ceric sulfate VS, determining the endpoint potentiometrically using a calomel-platinum electrode system. Each mL of 0.02 N ceric sulfate is equivalent to 4.221 mg of $C_{11}H_8Na_4O_8P_2$.

Menadiol Sodium Diphosphate Injection

» Menadiol Sodium Diphosphate Injection is a sterile solution of Menadiol Sodium Diphosphate in Water for Injection. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of $C_{11}H_8Na_4O_8P_2 \cdot 6H_2O$.

Packaging and storage—Preserve in single-dose, light-resistant containers, preferably of Type I glass.

USP Reference standards (11)—

USP Endotoxin RS

USP Menadione RS

Identification—

A: Transfer a volume of Injection, equivalent to about 100 mg of menadiol sodium diphosphate, to a separator, add 10 mL of 2 N sulfuric acid, and extract with six 25-mL portions of ether, discarding the ether extracts. To the aqueous solution add 1 mL of 0.5 N ceric sulfate and 1 mL of

30 percent hydrogen peroxide, and extract with two 10-mL portions of chloroform. Evaporate the combined chloroform extracts on a steam bath just to dryness, then dry at 80° for 1 hour: the IR absorption spectrum of a potassium bromide dispersion of the menadione so obtained exhibits maxima at the same wavelengths as that of a similar preparation of USP Menadione RS. The solid also responds to *Identification test B* under *Menadiol Sodium Diphosphate*.

B: Adjust, if necessary, a volume of Injection, equivalent to about 20 mg of menadiol sodium diphosphate, by evaporation or dilution with water, as required, to 2 mL: the solution responds to *Identification test C* under *Menadiol Sodium Diphosphate*.

Bacterial Endotoxins Test (85)—It contains not more than 25.0 USP Endotoxin Units per mg of menadiol sodium diphosphate.

pH (791): between 7.5 and 8.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of menadiol sodium diphosphate, to a 125-mL separator, and extract with three 25-mL portions of chloroform, discarding the chloroform extracts. Transfer the aqueous solution to a 250-mL beaker, add 25 mL of glacial acetic acid and 25 mL of 3 N hydrochloric acid, vigorously bubble nitrogen through this solution for not less than 15 minutes, and titrate with 0.01 N ceric sulfate VS, determining the endpoint potentiometrically using a calomel-platinum electrode system. Each mL of 0.01 N ceric sulfate is equivalent to 2.651 mg of $C_{11}H_8Na_4O_8P_2 \cdot 6H_2O$.

Menadiol Sodium Diphosphate Tablets

» Menadiol Sodium Diphosphate Tablets contain not less than 95.0 percent and not more than 110.0 percent of the labeled amount of $C_{11}H_8Na_4O_8P_2 \cdot 6H_2O$.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards (11)—

USP Menadione RS

Identification—

A: Triturate a quantity of powdered Tablets, equivalent to about 100 mg of menadiol sodium diphosphate, with a mixture of 10 mL of water and 10 mL of 2 N sulfuric acid, centrifuge the mixture, and filter the supernatant. To the filtrate add 1 mL of 0.5 N ceric sulfate, mix, extract with 10 mL of chloroform, and centrifuge. Evaporate the chloroform extract on a steam bath just to dryness, then dry at 80° for 1 hour: the IR absorption spectrum of a potassium bromide dispersion of the menadione so obtained exhibits maxima at the same wavelengths as that of a similar preparation of USP Menadione RS.

B: To 50 mg of the menadione obtained in *Identification test A* add 5 mL of water, then add 75 mg of sodium bisulfite, and heat on a steam bath, shaking vigorously until the substance is dissolved and the solution is almost colorless. Add water to make 50 mL, and mix. To 2 mL of the solution add 2 mL of alcoholic ammonia (prepared by mixing equal volumes of alcohol and ammonium hydroxide), shake, and add 3 drops of ethyl cyanoacetate: a deep purplish blue color is produced, and, on the addition of 1 mL of sodium hydroxide solution (1 in 3), it changes to green and then to yellow.

C: Triturate a quantity of powdered Tablets, equivalent to about 20 mg of menadiol sodium diphosphate, with 10 mL

of water, centrifuge the mixture, filter the supernatant, and evaporate to a volume of about 2 mL. Add 2 drops of nitric acid and 1 mL of sulfuric acid, and heat slowly to the evolution of white fumes. Cool, cautiously dilute with water to about 10 mL, and filter if not clear. Render the filtrate slightly alkaline to litmus with 6 N ammonium hydroxide, then render it acid with nitric acid, and add to the warm solution 3 mL of ammonium molybdate TS: a yellow precipitate is formed within a few minutes.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{11}H_8Na_4O_8P_2 \cdot 6H_2O$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 227 nm on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a standard solution prepared by dissolving in the same *Medium* an accurately weighed quantity of Menadiol Sodium Diphosphate, previously dried in vacuum over phosphorus pentoxide for 4 hours, the dried sample having a known concentration determined by titration with 0.01 N ceric sulfate VS as directed in the *Assay*.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{11}H_8Na_4O_8P_2 \cdot 6H_2O$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

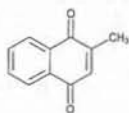
Procedure for content uniformity—[NOTE—Use low-actinic glassware.] Transfer 1 finely powdered Tablet to a glass-stoppered centrifuge tube, add 25 mL of pH 8.0 phosphate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*), and shake vigorously for several minutes. Filter into a 50-mL volumetric flask, rinse the centrifuge tube, and filter with three 5-mL portions of pH 8.0 phosphate buffer, adding the rinsings to the volumetric flask, dilute with pH 8.0 phosphate buffer to volume, and mix. Dilute a portion of this solution quantitatively and stepwise, if necessary, with pH 8.0 phosphate buffer to provide a solution containing approximately 40 µg of menadiol sodium diphosphate per mL. Concomitantly determine the absorbances of this solution and of a solution of Menadiol Sodium Diphosphate, previously dried in vacuum over phosphorus pentoxide for 4 hours, in the same *Medium* having a known concentration of about 40 µg per mL, at the wavelength of maximum absorbance at about 297 nm, with a suitable spectrophotometer, using pH 8.0 phosphate buffer as the blank. Calculate the quantity, in mg, of $C_{11}H_8Na_4O_8P_2 \cdot 6H_2O$ in the Tablet taken by the formula:

$$(TC/D)(A_U/A_S)$$

in which *T* is the labeled quantity, in mg, of menadiol sodium diphosphate in the Tablet, *C* is the concentration, in µg per mL, of $C_{11}H_8Na_4O_8P_2 \cdot 6H_2O$ in the Standard solution, *D* is the concentration, in µg per mL, of menadiol sodium diphosphate in the test solution, based upon the labeled quantity per Tablet and the extent of dilution, and *A_U* and *A_S* are the absorbances of the solution from the Tablet and the standard solution, respectively.

Assay—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of menadiol sodium diphosphate, to a 250-mL beaker. Moisten the powder with a few mL of glacial acetic acid, and then add sufficient quantity of the acid to make 25 mL. Add 25 mL of 3 N hydrochloric acid and 25 mL of water, mix, and titrate with 0.01 N ceric sulfate VS, determining the endpoint potentiometrically using a calomel-platinum electrode system. Each mL of 0.01 N ceric sulfate is equivalent to 2.651 mg of $C_{11}H_8Na_4O_8P_2 \cdot 6H_2O$.

Menadione



$C_{11}H_8O_2$ 172.18
1,4-Naphthalenedione, 2-methyl-;
2-Methyl-1,4-naphthoquinone [58-27-5].

DEFINITION

Menadione contains NLT 98.5% and NMT 101.0% of menadione ($C_{11}H_8O_2$), calculated on the dried basis.

[CAUTION—Menadione powder is irritating to the respiratory tract and to the skin, and a solution of it in alcohol is a vesicant.]

IDENTIFICATION

A. INFRARED ABSORPTION (197K)

B. ULTRAVIOLET ABSORPTION (197U)

Standard solution: 5 μ g/mL of USP Menadione RS in alcohol

Sample solution: 5 μ g/mL in alcohol

Analytical wavelength: 250 nm

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY

PROCEDURE

Sample solution: Transfer about 150 mg of Menadione into a 150-mL volumetric flask. Add 15 mL each of glacial acetic acid and 3 N hydrochloric acid, and rotate the flask until Menadione is dissolved. Add about 3 g of zinc dust, and close the flask with a stopper bearing a Bunsen valve. Shake, and allow to stand in the dark for 1 h, with frequent shaking. Rapidly decant the solution through a pledget of cotton into another flask, immediately wash the reduction flask with three 10-mL portions of freshly boiled and cooled water, and add 0.1 mL of orthophenanthroline TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N ceric sulfate VS

Endpoint detection: Potentiometric

Analysis: Immediately titrate the combined filtrate and washings with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N ceric sulfate is equivalent to 8.609 mg of menadione ($C_{11}H_8O_2$).

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

RESIDUE ON IGNITION (281): NMT 0.1%

ORDINARY IMPURITIES (466)

Standard solution and Sample solution: Methanol

Eluant: Chloroform

Visualization: 1

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

MELTING RANGE OR TEMPERATURE, Class I (741):

105°–107°

LOSS ON DRYING (731)

Analysis: Dry over silica gel for 4 h.

Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP REFERENCE STANDARDS (11)

USP Menadione RS

Menadione Injection

» Menadione Injection is a sterile solution of Menadione in oil. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{11}H_8O_2$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards (11)—

USP Endotoxin RS

USP Menadione RS

Bacterial Endotoxins Test (85)—It contains not more than 58.3 USP Endotoxin Units per mg of menadione.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—[NOTE—Avoid exposing Menadione and its solutions to light throughout the Assay.]

Standard preparation—Transfer about 25 mg of USP Menadione RS, accurately weighed, to a 100-mL volumetric flask, dissolve in a mixture of equal volumes of alcohol and ether, dilute with the same mixture to volume, and mix. Keep the solution tightly closed in a dark, cool place, and use it within 7 days.

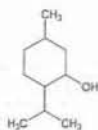
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 25 mg of menadione, to a 100-mL volumetric flask, dilute with a mixture of equal volumes of ether and alcohol to volume, and mix.

Procedure—Transfer 1.0 mL each of the *Standard preparation* and the *Assay preparation* to separate 50-mL volumetric flasks, add to each 4 mL of alcohol, and mix. Then to each flask add 1.0 mL of a solution prepared by dissolving 50 mg of 2,4-dinitrophenylhydrazine in 20 mL of a mixture of 2 volumes of 3 N hydrochloric acid and 1 volume of water. Place the flasks in a bath maintained at 70° to 75° for 15 minutes, shaking vigorously every 2 to 3 minutes. Immediately after the heating, cool the flasks to about 25°; then add to each 5 mL of alcoholic ammonia, prepared by mixing equal volumes of alcohol and ammonium hydroxide. Shake the flasks thoroughly, add alcohol to make 50.0 mL, mix, allow to stand for 15 minutes, and decant from any separated oil. Determine the absorbances of the solutions, in 1-cm cells at the wavelength of maximum absorbance at about 635 nm, with a suitable spectrophotometer, using a reagent blank to set the instrument. Calculate the quantity, in mg, of $C_{11}H_8O_2$ in each mL of the Injection taken by the formula:

$$(0.1C / V)(A_U / A_S)$$

in which C is the concentration, in μ g per mL, of USP Menadione RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Menthol



$C_{10}H_{20}O$ 156.27
Cyclohexanol, 5-methyl-2-(1-methylethyl)- [1490-04-6].

DEFINITION

Menthol is an alcohol obtained from oils derived from a variety of mints or prepared synthetically. Menthol may be levorotatory (*l*-menthol) from natural or synthetic sources, or racemic (*dl*-menthol). It contains NLT 98.0% and NMT 102.0% of menthol ($C_{10}H_{20}O$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the menthol peak of the *Standard solution*, as obtained in the *Assay*.
- **B.** It meets the requirements in *Specific Tests for Optical Rotation* (781S), *Specific Rotation*.

ASSAY

PROCEDURE

Internal standard solution: 0.5 mg/mL of anethole in hexanes

Standard solution: 0.5 mg/mL of USP Menthol RS in *Internal standard solution*

Sample solution: 0.5 mg/mL of Menthol in *Internal standard solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm \times 30-m fused silica; coated with a 1- μ m layer of G16 stationary phase

Temperatures

Injection port: 250°

Detector: 250°

Column: 130° (isothermal)

Carrier gas: Helium

Flow rate: 10 mL/min

Injection volume: 1 μ L

Injection type: Split ratio of 10:1

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for menthol and anethole are about 0.5 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.0 for the menthol peak

Relative standard deviation: NMT 2.0% for the peak response ratio of menthol to anethole in replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of menthol ($C_{10}H_{20}O$) in the portion of Menthol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of menthol to anethole from the *Sample solution*

R_S = peak response ratio of menthol to anethole from the *Standard solution*

C_S = concentration of USP Menthol RS in the *Standard solution* (mg/mL)

C_U = concentration of Menthol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

LIMIT OF NONVOLATILE RESIDUE

Analysis: Evaporate 2 g, accurately weighed, in a tared open porcelain dish on a steam bath, and dry the residue at 105° for 1 h.

Acceptance criteria: NMT 0.05%

RELATED COMPOUNDS

Internal standard solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Sample solution: 5 mg/mL of Menthol in hexanes

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual impurity in the portion of Menthol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the responses of all the peaks from the *Sample solution*

Acceptance criteria

Individual impurities: NMT 0.3%

Total impurities: NMT 2.0%

READILY OXIDIZABLE SUBSTANCES IN *dl*-MENTHOL

Sample solution: Place 500 mg of *dl*-menthol in a clean, dry test tube, and add 10 mL of a solution of potassium permanganate, prepared by diluting 3 mL of 0.1 N potassium permanganate with water to 100 mL.

Analysis: Place the test tube in a beaker with water at a temperature between 45° and 50°. Remove the tube from the bath at intervals of 30 s, and mix quickly by shaking.

Acceptance criteria: The purple color of potassium permanganate is still apparent after 5 min.

SPECIFIC TESTS

CONGEALING RANGE OF *dl*-MENTHOL

(See *Congeeing Temperature* (651).)

[NOTE—Perform this test preferably in a room having a temperature below 30° and a relative humidity below 50%.]

Sample: 10 g of *dl*-menthol, previously dried in a desiccator over silica gel for 24 h

Analysis: Place the *Sample* in a dry test tube having an internal diameter of 18–20 mm, and melt the contents at a temperature of about 40°. Suspend the test tube in water having a temperature of 23°–25°, and stir the contents of the tube continually with a thermometer, keeping the bulb of the thermometer immersed in the liquid.

Acceptance criteria: *dl*-Menthol congeals at a temperature between 27° and 28°. Shortly after the temperature has stabilized at the congealing point, add a few mg of dried *dl*-menthol to the congealed mass, and continue stirring. After a few min, the temperature of the mass quickly rises to 30.5°–32.0°.

MELTING RANGE OF *l*-MENTHOL

(See *Melting Range or Temperature* (741).)

Acceptance criteria: 41°–44°

OPTICAL ROTATION (781S), *Specific Rotation*

Sample solution: 100 mg/mL in alcohol

Acceptance criteria

l-Menthol: –45° to –51°

dl-Menthol: –2° to +2°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, preferably at controlled room temperature.

• **LABELING:** Label it to indicate whether it is levorotatory or racemic.

- **USP REFERENCE STANDARDS (11)**
USP Menthol RS

Menthol Lozenges

DEFINITION

Menthol Lozenges contain NLT 90.0% and NMT 125.0% of the labeled amount of menthol ($C_{10}H_{20}O$), in a suitable molded base.

IDENTIFICATION

- **A.** The retention time of the menthol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: 250 mg/mL of sodium chloride in water
Internal standard solution: 2 mg/mL of anethole in hexanes

Standard solution: 0.20L mg/mL of USP Menthol RS in *Internal standard solution*, where *L* is the labeled quantity, in mg, of menthol in each Lozenge

Sample solution: Transfer 20 Lozenges to a 1-L screw-capped conical flask. [NOTE—Use caps with inert white rubber liners.] Add 200 mL of water, 260 mL of *Solution A*, and 100.0 mL of the *Internal standard solution*, and shake by mechanical means for 30 min. Allow the phases to separate, and transfer a portion of the hexanes phase to a suitable container.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm \times 30-m fused silica; coated with a 1- μ m layer of G16 stationary phase

Temperatures

Column: 125° (isothermally)

Injection port: 250°

Detector: 250°

Carrier gas: Helium

Flow rate: 10 mL/min

Injection volume: 1 μ L

Injection type: Split ratio of 10:1

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for menthol and anethole are about 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 15 between menthol and anethole

Tailing factor: NMT 2.0 for menthol and anethole

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of menthol ($C_{10}H_{20}O$) in the portion of Lozenges taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the menthol to the anethole from the *Sample solution*

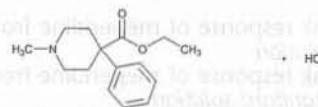
R_S = peak response ratio of the menthol to the anethole from the *Standard solution*
 C_S = concentration of USP Menthol RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of menthol in the hexanes phase of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–125.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
USP Menthol RS

Meperidine Hydrochloride



$C_{15}H_{21}NO_2 \cdot HCl$ 283.79
4-Piperidinecarboxylic acid, 1-methyl-4-phenyl-, ethyl ester, hydrochloride;
Ethyl 1-methyl-4-phenylisopiecotate hydrochloride [50-13-5].

DEFINITION

Meperidine Hydrochloride contains NLT 98.0% and NMT 102.0% of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181):**
Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL, Chloride (191)**
Sample solution: 10 mg/mL
Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: Transfer about 6.8 g of monobasic potassium phosphate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Add 10 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 7.0, and filter.

Mobile phase: Acetonitrile and *Solution A* (550:450), filtered and degassed

Standard stock solution: 0.6 mg/mL of USP Meperidine Hydrochloride RS in water

Standard solution: 0.12 mg/mL of USP Meperidine Hydrochloride RS from the *Standard stock solution* in *Mobile phase*

Sample stock solution: 0.6 mg/mL of Meperidine Hydrochloride in water

Sample solution: 0.12 mg/mL of Meperidine Hydrochloride from the *Sample stock solution* in *Mobile phase*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitabilitySample: *Standard solution***Suitability requirements**

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2 for the meperidine peak

Relative standard deviation: NMT 2%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$) in the portion of Meperidine Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of meperidine from the *Sample solution*

r_s = peak response of meperidine from the *Standard solution*

C_s = concentration of USP Meperidine Hydrochloride RS in the *Standard solution* (mg/mL)

C_u = concentration of Meperidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

OTHER COMPONENTS**• CONTENT OF CHLORIDE**

Sample solution: Transfer about 500 mg of Meperidine Hydrochloride, previously dried, to a 250-mL conical flask. Add 15 mL of water, 5 mL of glacial acetic acid, 50 mL of methanol, and 0.2 mL of eosin Y TS.

Analysis: Titrate the *Sample solution* with 0.1 N silver nitrate VS to a rose-colored endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.

Acceptance criteria: 12.2%–12.7% of chloride is found.

IMPURITIES**• RESIDUE ON IGNITION (281):** NMT 0.1%**• ORGANIC IMPURITIES**

Sample solution: 10 mg/mL in water

Chromatographic system

Mode: GC

Detector: Flame ionization

Column: 2-mm × 2-m glass; 10% phase G3 on support S1A

Temperatures

Column: 190°

Injection port: 255°

Detector: 280°

Carrier gas: Helium

Flow rate: 28 mL/min

Injection volume: 2.0 µL

AnalysisSample: *Sample solution*

Calculate the area percentage of each peak.

Acceptance criteria: No peak other than the principal peak (except for the solvent peak) constitutes more than 1.0% of the total area.

SPECIFIC TESTS**• LOSS ON DRYING (731)**

Analysis: Dry under vacuum at a pressure between 20 and 40 mm of mercury at 80° for 4 h.

Acceptance criteria: NMT 1.0%

• MELTING RANGE OR TEMPERATURE (741)

Sample: Dried under vacuum at 80° for 4 h

Acceptance criteria: 186°–189°

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in well-closed, light-resistant containers, and store at room temperature.

• USP REFERENCE STANDARDS (11)

USP Meperidine Hydrochloride RS

Meperidine Hydrochloride Injection**DEFINITION**

Meperidine Hydrochloride Injection is a sterile solution of Meperidine Hydrochloride in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$).

IDENTIFICATION**• A. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181):**

Meets the requirements

• B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY**• PROCEDURE**

Buffer: Transfer about 6.8 g of monobasic potassium phosphate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Add 10 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 7.0, and filter.

Mobile phase: Acetonitrile and *Buffer* (550:450), filtered and degassed

Standard stock solution: 0.6 mg/mL of USP Meperidine Hydrochloride RS in water

Standard solution: 0.12 mg/mL of USP Meperidine Hydrochloride RS from the *Standard stock solution* in *Mobile phase*

Sample stock solution: Transfer a measured volume of Injection equivalent to about 300 mg to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 1.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitabilitySample: *Standard solution***Suitability requirements**

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2

Relative standard deviation: NMT 2%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Meperidine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of meperidine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

- **PH (791):** 3.5–6.0
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 2.4 USP Endotoxin Units/mg of meperidine hydrochloride.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS (11)**
USP Endotoxin RS
USP Meperidine Hydrochloride RS

Meperidine Hydrochloride Oral Solution

DEFINITION

Meperidine Hydrochloride Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$).

IDENTIFICATION

- **A.**
Sample solution: Transfer a volume of Oral Solution nominally equivalent to about 100 mg of meperidine hydrochloride to a 125-mL separator. Add 40 mL of water and 3 mL of 1 N sodium hydroxide, and extract with three 25-mL portions of *n*-hexane. Wash the combined extracts with two 20-mL portions of water, discard the water, and then extract with three 25-mL portions of 0.1 N hydrochloric acid. Transfer the extracts to a 100-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.
Standard solution: Prepare in a way similar to that for the *Sample solution*, using USP Meperidine Hydrochloride RS.
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*.

ASSAY

• PROCEDURE

- Sample solution:** Transfer a suitable volume of Oral Solution nominally equivalent to about 250 mg of meperidine hydrochloride to a separator, and add 3 mL of 1 N sodium hydroxide. Extract with five 20-mL portions of chloroform, and filter the extracts through a pledget of cotton into a 250-mL conical flask. Wash the cotton with 5 mL of chloroform, and add the washing to the combined filtrates. Add 10 mL of glacial acetic acid and 2 drops of crystal violet TS.
Analysis: Titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 28.38 mg of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$).
Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DELIVERABLE VOLUME (698):** Meets the requirements for oral solution packaged in multiple-unit containers

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements for oral solution packaged in single-unit containers

SPECIFIC TESTS

- **PH (791):** 3.5–4.1

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**
USP Meperidine Hydrochloride RS

Meperidine Hydrochloride Tablets

DEFINITION

Meperidine Hydrochloride Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$).

IDENTIFICATION

• A. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181)

Sample solution: Transfer an amount nominally equivalent to about 50 mg of meperidine hydrochloride from powdered Tablets to a separator, add 10 mL of water, and shake. Add 5 mL of saturated sodium chloride solution and 1 mL of sodium hydroxide solution (1 in 25). Extract with three 20-mL portions of chloroform, filtering the extracts through cotton overlaid with anhydrous sodium sulfate. Evaporate the chloroform on a steam bath, and dissolve the residue in 4 mL of carbon disulfide.

Standard solution: In a second separator, proceed as directed in the *Sample solution*, using 50 mg of USP Meperidine Hydrochloride RS.

Analysis: Proceed as directed in the chapter, beginning with "Determine the absorption spectra".

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: Transfer about 6.8 g of monobasic potassium phosphate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Add 10 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 7.0, and filter.

Mobile phase: Acetonitrile and *Solution A* (550:450), filtered and degassed

Standard stock solution: 0.6 mg/mL of USP Meperidine Hydrochloride RS in water

Standard solution: 0.12 mg/mL of USP Meperidine Hydrochloride RS from the *Standard stock solution* in *Mobile phase*

Sample stock solution: Transfer an amount nominally equivalent to about 60 mg of meperidine hydrochloride from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add about 70 mL of *Mobile phase*, and sonicate for 10 min with occasional shaking. Shake by mechanical means for about 30 min, dilute with *Mobile phase* to volume, mix, and filter.

Sample solution: Nominally equivalent to 0.12 mg/mL of meperidine hydrochloride in *Mobile phase* from the *Sample stock solution*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitabilitySample: *Standard solution***Suitability requirements**

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2 for the meperidine peak

Relative standard deviation: NMT 2%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of meperidine from the *Sample solution* r_S = peak response of meperidine from the *Standard solution* C_S = concentration of USP Meperidine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = nominal concentration of meperidine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS**• DISSOLUTION (711)**

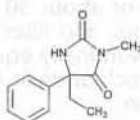
Medium: Water; 500 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: A known concentration of USP Meperidine Hydrochloride RS in *Medium**Sample solution*: Filter portions of the solution under test, and suitably dilute with *Medium*, if necessary.**Chromatographic system and System suitability**: Proceed as directed in the *Assay*.**Analysis**: Determine the labeled amount of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$) dissolved by comparing the peak response of meperidine from the *Sample solution* with that from the *Standard solution*.**Tolerances**: NLT 75% (Q) of the labeled amount of meperidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$) is dissolved.**• UNIFORMITY OF DOSAGE UNITS (905)**: Meet the requirements**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE**: Preserve in well-closed, light-resistant containers.**• USP REFERENCE STANDARDS (11)**

USP Meperidine Hydrochloride RS

Mephenytoin $C_{12}H_{14}N_2O_2$

218.25

2,4-Imidazolidinedione, 5-ethyl-3-methyl-5-phenyl-, (±)-; (±)-5-Ethyl-3-methyl-5-phenylhydantoin [50-12-4].

DEFINITIONMephenytoin contains NLT 98.0% and NMT 102.0% of mephenytoin ($C_{12}H_{14}N_2O_2$), calculated on the dried basis.**IDENTIFICATION****• A. INFRARED ABSORPTION (197K)****• B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution* as obtained in the *Assay*.**ASSAY****• PROCEDURE***Mobile phase*: Acetonitrile, methanol, and water (10:38:52)*System suitability solution*: 0.015 mg/mL of propiophenone and 1.5 mg/mL of USP Mephenytoin RS in *Mobile phase*. Sonicate if necessary.*Standard solution*: 5.0 mg/mL of USP Mephenytoin RS in *Mobile phase*. Sonicate if necessary.*Sample solution*: 5.0 mg/mL of Mephenytoin in *Mobile phase***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 257 nm

Column: 3.9-mm × 15-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitabilitySample: *System suitability solution*[NOTE—See *Table 1* for relative retention times.]**Suitability requirements**

Column efficiency: NLT 4000 theoretical plates for the mephenytoin peak

Relative standard deviation: NMT 2.0% for the mephenytoin peak

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of mephenytoin ($C_{12}H_{14}N_2O_2$) in the portion of Mephenytoin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Mephenytoin RS in the *Standard solution* (mg/mL) C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES**• RESIDUE ON IGNITION (281)**: NMT 0.1%**Delete the following:****• HEAVY METALS, Method II (231)**: NMT 20 ppm (Official 1; Jan-2018)**• ORGANIC IMPURITIES***Mobile phase*: Acetonitrile, methanol, and water (10:38:52)*System suitability solution*: 0.015 mg/mL of propiophenone and 1.5 mg/mL of USP Mephenytoin RS in *Mobile phase**Sample solution*: 5.0 mg/mL of Mephenytoin in *Mobile phase*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 225 nm**Column:** 3.9-mm × 15-cm; packing L7**Flow rate:** 1 mL/min**Injection volume:** 10 µL**System suitability****Sample:** *System suitability solution*[NOTE—See *Table 1* for relative retention times.]**Suitability requirements****Column efficiency:** NLT 4000 theoretical plates for the mephenytoin peak**Relative standard deviation:** NMT 2.0% for the mephenytoin peak**Analysis****Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Mephenytoin taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

 r_U = peak response for each impurity r_T = sum of the responses of all of the peaks F = relative response factor for the corresponding impurity (see *Table 1*)**Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desmethyl phenytoin ^a	0.66	0.86	1.0
Mephenytoin	1.0	—	—
Methyl mephenytoin ^b	1.17	1.0	1.0
Propiopnone	1.5	2.7	1.0
Any individual unspecified impurity	—	1.0	0.10
Total	—	—	1.5

^a 5-Ethyl-5-phenylimidazolidine-2,4-dione.^b 5-Ethyl-1,3-dimethyl-5-phenylimidazolidine-2,4-dione.**SPECIFIC TESTS****• LOSS ON DRYING (731)****Analysis:** Dry at 105° for 4 h.**Acceptance criteria:** NMT 1.0%**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight containers below 30°.**• USP REFERENCE STANDARDS (11)**

USP Mephenytoin RS

Mephenytoin Tablets**DEFINITION**Mephenytoin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of mephenytoin (C₁₂H₁₄N₂O₂).**IDENTIFICATION****• A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**ASSAY****• PROCEDURE****Mobile phase:** Acetonitrile, methanol, and water (10:38:52)**System suitability solution:** 0.015 mg/mL of propiopnone and 1.5 mg/mL of USP Mephenytoin RS in *Mobile phase*. Sonicate if necessary.**Standard solution:** 5.0 mg/mL of USP Mephenytoin RS in *Mobile phase*. Sonicate if necessary.**Sample solution:** Nominally 5.0 mg/mL of mephenytoin prepared with NLT 500 mg from NLT 20 powdered Tablets as follows. Transfer the powder to a suitable volumetric flask. Add 60% of the flask volume of *Mobile phase*, sonicate for 10 min, and shake by mechanical means for 30 min. Dilute with *Mobile phase* to volume, and filter, discarding a suitable portion of the filtrate.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 257 nm**Column:** 3.9-mm × 15-cm; packing L7**Flow rate:** 1 mL/min**Injection volume:** 10 µL**System suitability****Sample:** *System suitability solution*[NOTE—See *Table 1* for relative retention times.]**Suitability requirements****Column efficiency:** NLT 4000 theoretical plates for the mephenytoin peak**Relative standard deviation:** NMT 2.0% for the mephenytoin peak**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of mephenytoin (C₁₂H₁₄N₂O₂) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Mephenytoin RS in the *Standard solution* (mg/mL) C_U = nominal concentration of mephenytoin in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS****• DISSOLUTION (711)****Medium:** Water; 500 mL**Apparatus 2:** 75 rpm**Time:** 60 min**Instrumental conditions****Mode:** UV**Analytical wavelength:** Wavelength of maximum absorbance at about 257 nm**Standard solution:** 0.2 mg/mL of USP Mephenytoin RS in *Medium***Sample solution:** Filter a portion of the solution under test. Dilute the filtrate, if necessary, with *Medium*.**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of mephenytoin (C₁₂H₁₄N₂O₂) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of USP Mephenytoin RS in the *Standard solution* (mg/mL) D = dilution factor, if used V = volume of *Medium*, 500 mL L = label claim (mg/Tablet)

Tolerances: NLT 70% (Q) of the labeled amount of mephenytoin ($C_{12}H_{14}N_2O_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase: Acetonitrile, methanol, and water (10:38:52)

System suitability solution: 0.015 mg/mL of propiophenone and 1.5 mg/mL of USP Mephenytoin RS in *Mobile phase*. Sonicate if necessary.

Sample solution: Nominally 5.0 mg/mL of mephenytoin prepared with NLT 500 mg from NLT 20 powdered Tablets as follows. Transfer the powder to a suitable volumetric flask. Add 60% of the flask volume of *Mobile phase*, sonicate for 10 min, and shake by mechanical means for 30 min. Dilute with *Mobile phase* to volume, and filter, discarding a suitable portion of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 3.9-mm × 15-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Column efficiency: NLT 4000 theoretical plates for the mephenytoin peak

Relative standard deviation: NMT 2.0% for the mephenytoin peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_T) \times (1/F) \times 100$$

r_u = peak response of each impurity

r_T = sum of the responses of all of the peaks

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desmethyl phenytoin ^a	0.66	0.86	1.0
Mephenytoin	1.0	—	—
Methyl mephenytoin ^b	1.2	1.0	1.0
Propiophenone	1.5	2.7	1.0
Any individual unspecified degradation product	—	1.0	0.2
Total impurities	—	—	2.0

^a 5-Ethyl-5-phenylimidazolidine-2,4-dione.

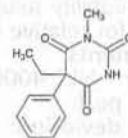
^b 5-Ethyl-1,3-dimethyl-5-phenylimidazolidine-2,4-dione.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store below 30°.

- **USP REFERENCE STANDARDS (11)**
USP Mephenytoin RS

Mephobarbital



$C_{13}H_{14}N_2O_3$ 246.26
2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-1-methyl-5-phenyl-;
5-Ethyl-1-methyl-5-phenylbarbituric acid [115-38-8].

DEFINITION

Mephobarbital contains NLT 98.0% and NMT 100.5% of mephobarbital ($C_{13}H_{14}N_2O_3$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**

- **B.**

Sample: 200 mg of Mephobarbital

Analysis: Boil the *Sample* with 10 mL of 1 N sodium hydroxide.

Acceptance criteria: Ammonia is evolved.

- **C.**

Diluent: Sodium hydroxide solution (1 in 500)

Sample solution: 12 mg/mL of Mephobarbital prepared as follows. Shake about 60 mg of Mephobarbital with 5 mL of *Diluent*, and filter. Use the filtrate.

Analysis 1: Add 3 drops of mercuric nitrate TS to 1 mL of the *Sample solution*.

Acceptance criteria 1: A white precipitate is formed, and it is soluble in 6 N ammonium hydroxide.

Analysis 2: Add silver nitrate TS to 1 mL of the *Sample solution*.

Acceptance criteria 2: A white precipitate is formed, and it dissolves readily in 6 N ammonium hydroxide.

ASSAY

- **PROCEDURE**

Sample solution: 10 mg/mL of Mephobarbital in dimethylformamide

Analysis: Transfer 50 mL of *Sample solution* to a 200-mL flask. Add 4 drops of thymolphthalein TS. Titrate with 0.1 N lithium methoxide in toluene VS using a magnetic stirrer and a cover for the flask to protect against atmospheric carbon dioxide. Perform a blank determination. Each mL of 0.1 N lithium methoxide is equivalent to 24.63 mg of mephobarbital ($C_{13}H_{14}N_2O_3$).

Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

SPECIFIC TESTS

- **LOSS ON DRYING (731)**

Analysis: Dry a sample at 105° for 4 h.

Acceptance criteria: NMT 1.0%

- **MELTING RANGE OR TEMPERATURE, Class I (741):** 176°–181°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**
USP Mephobarbital RS

Mephobarbital Tablets

DEFINITION

Mephobarbital Tablets contain NLT 95.0% and NMT 110.0% of the labeled amount of mephobarbital ($C_{13}H_{14}N_2O_3$).

IDENTIFICATION

- **A. INFRARED ABSORPTION—GENERAL (197M)**
Sample: Residue obtained in the Assay
Acceptance criteria: The infrared absorption spectrum of Sample is consistent with that of USP Mephobarbital RS.
- **B. MELTING RANGE OR TEMPERATURE (741)**
Sample: Residue obtained in the Assay
Acceptance criteria: 174°–181°

ASSAY

• PROCEDURE

Sample: Weigh a suitable number of powdered Tablets (NLT 20), and transfer an accurately weighed portion of powder, equivalent to 300 mg of mephobarbital, to a suitable extraction thimble. Extract with 15 mL of hexane, allow the thimble to drain, transfer to a continuous-extraction apparatus provided with a tared flask, and extract the mephobarbital with chloroform for 2 h. Evaporate the chloroform on a steam bath with the aid of a current of air, and cool.

Analysis: Dissolve the residue in 10 mL of alcohol, and evaporate. Dry the residue at 105°; for 1 h, cool, and weigh. The weight of the residue represents the weight of mephobarbital ($C_{13}H_{14}N_2O_3$) in the portion of Tablets taken.

Acceptance criteria: 95.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Buffer: 1 L of 1% of 3-(dodecyldimethylammonio) propanesulfonate in pH 8.0 phosphate buffer prepared as follows. Transfer 10.0 g of 3-(dodecyldimethylammonio) propanesulfonate in 400 mL of warm water, and add 250 mL of 0.2 M monobasic potassium phosphate and about 220 mL of 0.2 M sodium hydroxide. Cool to room temperature, adjust with 0.2 M sodium hydroxide to a pH of 8.0, dilute with water to 1000 mL, mix, and degas.

Medium: Buffer; 900 mL

Apparatus 2: 75 rpm

Time: 75 min

Standard solution: Known concentration of USP Mephobarbital RS in Medium

Sample solution: Pass a portion of the solution through a nylon filter of 0.45- μ m pore size, and, if necessary, suitably dilute with Medium.

Instrumental conditions

Mode: UV

Analytical wavelength: 244 nm

Analysis

Samples: Standard solution and Sample solution

Tolerances: NLT 70% (Q) of the labeled amount of mephobarbital ($C_{13}H_{14}N_2O_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS, Content Uniformity (905)**
Prepare all of the solutions concomitantly.

Diluent: 6.2 g of boric acid and 7.45 g of potassium chloride in 500 mL of water. Add 210 mL of sodium hydroxide solution (1 in 25). Add water to make 2000 mL of Diluent.

Standard stock solution: 10 mg/mL of USP Mephobarbital RS in Diluent

Standard solution: 1.5 mg/mL of USP Mephobarbital RS in water from Standard stock solution

Sample solution: Nominally 1.5 mg/mL of mephobarbital prepared as follows. Transfer 1 Tablet to a glass-stoppered centrifuge tube, crush the Tablet, and add 25.0 mL of Diluent. Insert the stopper, shake for 10 min, and, if necessary, centrifuge until clear, filtering the supernatant. Dilute a portion of the subsequent liquid with water.

Instrumental conditions

Mode: UV

Analytical wavelength: 245 nm

Cell: 1 cm

Blank: Diluent and water (1:3)

Analysis

Samples: Standard solution and Sample solution

Transfer 3.0 mL each of the Standard solution and the Sample solution to separate 200-mL volumetric flasks, and dilute each with Blank to volume.

Determine the percentage of the labeled amount of mephobarbital ($C_{13}H_{14}N_2O_3$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the Sample solution

A_S = absorbance of the Standard solution

C_S = concentration of the USP Mephobarbital RS in the Standard solution (mg/mL)

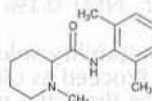
C_U = nominal concentration of mephobarbital in the Sample solution (mg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
USP Mephobarbital RS

Mepivacaine Hydrochloride



$C_{15}H_{22}N_2O \cdot HCl$ 282.81
2-Piperidinecarboxamide, N-(2,6-dimethylphenyl)-1-methyl-, monohydrochloride, (\pm);
(\pm)-1-Methyl-2',6'-pipecoloxylidide monohydrochloride [1722-62-9].

DEFINITION

Mepivacaine Hydrochloride contains NLT 98.0% and NMT 102.0% of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride (191)**
Sample solution: 10 mg/mL
Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Buffer: 2.25 g/L solution of phosphoric acid, adjusted with 50% sodium hydroxide to a pH of 7.6

Mobile phase: Acetonitrile and Buffer (35:65)
System suitability solution: 2 µg/mL of USP Mepivacaine Hydrochloride RS and 3 µg/mL of USP Bupivacaine Related Compound B RS in *Mobile phase*
Standard solution: 0.2 mg/mL of USP Mepivacaine Hydrochloride RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Mepivacaine Hydrochloride in *Mobile phase*
Chromatographic system
 (See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.5 between bupivacaine related compound B and mepivacaine, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$) in the portion of Mepivacaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mepivacaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Mepivacaine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*. The run time is three times the retention time of the mepivacaine peak.

Standard solution: 2 µg/mL of USP Mepivacaine Hydrochloride RS in *Mobile phase*

Sample solution: 2 mg/mL of Mepivacaine Hydrochloride in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.5 between bupivacaine related compound B and mepivacaine, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any impurity in the portion of Mepivacaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of mepivacaine from the *Standard solution*

C_S = concentration of USP Mepivacaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Mepivacaine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bupivacaine related compound B (desmethyl mepivacaine) ^a	0.4	0.15
Mepivacaine	1.0	—
Picolinamide analog ^b	2.1	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	0.4

^a N-(2,6-Dimethylphenyl)piperidine-2-carboxamide.

^b N-(2,6-Dimethylphenyl)picolinamide.

2,6-DIMETHYLANILINE

Prepare the *Standard solution* and *Sample solution* fresh, just before use.

Standard stock solution: 0.6 µg/mL of USP Ropivacaine Related Compound A RS in 1 N hydrochloric acid. [NOTE—Ropivacaine related compound A is 2,6-dimethylaniline hydrochloride.]

Standard solution: Transfer 2.0 mL of the *Standard stock solution* and 1.0 mL of 3 N sodium hydroxide to a 20-mL headspace vial, and immediately close the vial with a cap.

Sample stock solution: 30 mg/mL of Mepivacaine Hydrochloride in 1 N hydrochloric acid

Sample solution: Transfer 2.0 mL of the *Sample stock solution* and 1.0 mL of 3 N sodium hydroxide to a 20-mL headspace vial, and immediately close the vial with a cap.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m capillary; coated with 3-µm film of G43

Temperatures

Injection port: 225°

Detector: 250°

Column: See Table 2.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	10	230	5

Carrier gas: Helium
 Flow rate: 4 mL/min
 Injection type: Split ratio, 1:1
 Headspace sampler
 Equilibration time: 15 min
 Equilibration temperature: 90°
 Loop temperature: 215°
 Transfer line temperature: 220°
 Vial pressure: About 15 psi
 Loop size: 3 mL
 System suitability
 Sample: *Standard solution*
 Suitability requirements
 Tailing factor: NMT 2.0
 Relative standard deviation: NMT 15%

Analysis

Samples: *Standard solution* and *Sample solution*
 Calculate the quantity, in ppm, of 2,6-dimethylaniline in the portion of Mepivacaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 10^6$$

r_U = peak response of 2,6-dimethylaniline from the *Sample solution*

r_S = peak response of 2,6-dimethylaniline from the *Standard solution*

C_S = concentration of USP Ropivacaine Related Compound A RS in the *Standard solution* (µg/mL)

C_U = concentration of Mepivacaine Hydrochloride in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of 2,6-dimethylaniline, 121.18

M_{r2} = molecular weight of 2,6-dimethylaniline hydrochloride (ropivacaine related compound A), 157.64

Acceptance criteria: NMT 20 ppm

SPECIFIC TESTS• **LOSS ON DRYING** (731)

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** (11)

USP Bupivacaine Related Compound B RS
 N-(2,6-Dimethylphenyl)piperidine-2-carboxamide.

$C_{14}H_{20}N_2O$ 232.32

USP Mepivacaine Hydrochloride RS

USP Ropivacaine Related Compound A RS

2,6-Dimethylaniline hydrochloride.

$C_8H_{11}N \cdot HCl$ 157.64

Mepivacaine Hydrochloride Injection**DEFINITION**

Mepivacaine Hydrochloride Injection is a sterile solution of Mepivacaine Hydrochloride in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$).

IDENTIFICATION

• **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** (181): Meets the requirements

• **B. Analysis:** Extract a volume of Injection, equivalent to 200 mg of mepivacaine, with two 10-mL portions of ether, and discard the ether extracts. Render the remaining solution slightly alkaline with sodium carbonate

TS, and extract the precipitate with ether. Evaporate the ether extract on a steam bath to dryness, and dry the residue under vacuum at 60° for 1 h.

Acceptance criteria: The mepivacaine obtained melts between 149° and 153°.

ASSAY• **PROCEDURE**

Buffer: 3.40 g/L of monobasic potassium phosphate and 4.35 g/L of dibasic potassium phosphate in water. Adjust with potassium hydroxide or phosphoric acid to a pH of 6.3.

Mobile phase: Acetonitrile and *Buffer* (35:65)

System suitability solution: 0.05 mg/mL of methylparaben and 1.0 mg/mL of USP Mepivacaine Hydrochloride RS in *Mobile phase*

Standard solution: 1.0 mg/mL of USP Mepivacaine Hydrochloride RS in *Mobile phase*

Sample solution: Nominally 1 mg/mL of mepivacaine hydrochloride from Injection in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 263 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1[†]

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for mepivacaine and methylparaben are 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 2.0 between methylparaben and mepivacaine, *System suitability solution*

Capacity factor: NLT 1.0 for the mepivacaine peak, *System suitability solution*

Tailing factor: NMT 2.0 for the mepivacaine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$) in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mepivacaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

• **pH** (791): 4.5–6.8

• **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 0.8 USP Endotoxin Unit/mg of mepivacaine hydrochloride.

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Injection labeled to contain 2% or less of mepivacaine hydrochloride may be packaged in 50-mL multiple-dose containers.

[†] A Whatman Partisphere RTF C18 brand of L1 column has been shown to be an appropriate column.

• **USP REFERENCE STANDARDS (11)**

- USP Endotoxin RS
- USP Mepivacaine Hydrochloride RS

Mepivacaine Hydrochloride and Levonordefrin Injection

DEFINITION

Mepivacaine Hydrochloride and Levonordefrin Injection is a sterile solution of Mepivacaine Hydrochloride and Levonordefrin in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$) and NLT 90.0% and NMT 110.0% of the labeled amount of levonordefrin ($C_9H_{13}NO_3$).

IDENTIFICATION

- A.**
Analysis: Extract a volume of Injection, equivalent to 200 mg of mepivacaine, with two 10-mL portions of ether, and discard the ether extracts. Render slightly alkaline with sodium carbonate TS, extract the precipitate with ether, evaporate the ether extract on a steam bath to dryness, and dry the residue under vacuum at 60° for 1 h.
Acceptance criteria: The mepivacaine obtained melts between 149° and 153°.
- B. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements

ASSAY

• MEPIVACAINE HYDROCHLORIDE

Buffer: 3.40 g/L of monobasic potassium phosphate and 4.35 g/L of dibasic potassium phosphate in water. Adjust with potassium hydroxide or phosphoric acid to a pH of 6.3.

Mobile phase: Acetonitrile and Buffer (35:65)

System suitability solution: 0.05 mg/mL of methylparaben and 1.0 mg/mL of USP Mepivacaine Hydrochloride RS in Mobile phase

Standard solution: 1.0 mg/mL of USP Mepivacaine Hydrochloride RS in Mobile phase

Sample solution: Nominally 1 mg/mL of mepivacaine hydrochloride from Injection in Mobile phase

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 263 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1¹

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: System suitability solution and Standard solution

[NOTE—The relative retention times for mepivacaine and methylparaben are 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 2.0 between methylparaben and mepivacaine, System suitability solution

Capacity factor: NLT 1.0 for the mepivacaine peak, System suitability solution

Tailing factor: NMT 2.0 for the mepivacaine peak, System suitability solution

Relative standard deviation: NMT 2.0%, Standard solution

Analysis

Samples: Standard solution and Sample solution
 Calculate the percentage of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$) in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the Sample solution
- r_S = peak response from the Standard solution
- C_S = concentration of USP Mepivacaine Hydrochloride RS in the Standard solution (mg/mL)
- C_U = nominal concentration of mepivacaine hydrochloride in the Sample solution (mg/mL)

Acceptance criteria: 95.0%–105.0%

• LEVONORDEFRIN

Ferro-citrate solution and Buffer solution: Prepare as directed in Epinephrine Assay (391).

Standard stock solution: With the aid of 20 mL of sodium bisulfite solution (1 in 50), transfer 25 mg of USP Levonordefrin RS to a 50-mL volumetric flask, and dilute with water to volume.

Standard solution: 50 μg/mL of USP Levonordefrin RS in sodium bisulfite solution (1 in 500) from the Standard stock solution. Make the final dilution at the time the Assay is to be carried out.

Sample solution: Nominally 50 μg/mL of levonordefrin from Injection, diluting if necessary

Analysis: Proceed as directed in Epinephrine Assay (391), except use levonordefrin wherever epinephrine [base] is called for. When the Ferro-citrate solution and the Buffer solution are mixed with the Sample solution, a fine precipitate may be formed. Remove this precipitate by centrifugation or by passing through dry filter paper before the colorimetric measurements are taken.

Calculate the percentage of the labeled amount of levonordefrin ($C_9H_{13}NO_3$) in the volume of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

- A_U = absorbance of the Sample solution
- A_S = absorbance of the Standard solution
- C_S = concentration of USP Levonordefrin RS in the Standard solution (μg/mL)
- C_U = nominal concentration of levonordefrin in the Sample solution (μg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• COLOR AND CLARITY

Standard solution: Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, and dilute with water to volume.

Analysis

Samples: Standard solution and Sample solution

Visually examine a portion of the Injection (Sample solution) in a suitable clear glass test tube against a white background: it is not pinkish, and it contains no precipitate. If any yellow color is observed in the Sample solution, concomitantly determine the absorbances of the Sample solution and Standard solution in 1-cm cells with a suitable spectrophotometer set at 460 nm.

Acceptance criteria: The absorbance of the Sample solution does not exceed that of the Standard solution.

• PH (791): 3.3–5.5

• **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.8 USP Endotoxin Unit/mg of mepivacaine hydrochloride.

• **OTHER REQUIREMENTS:** It meets the requirements in Injections and Implanted Drug Products (1).

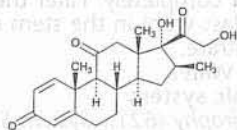
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

¹ A Whatman Partisphere RTF C18 brand of L1 column has been shown to be an appropriate column.

- **LABELING:** The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.
- **USP REFERENCE STANDARDS (11)**
 - USP Endotoxin RS
 - USP Levonordefrin RS
 - USP Mepivacaine Hydrochloride RS

Meprednisone



$C_{22}H_{28}O_5$ 372.45
 Pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-16-methyl-, (16 β)-;
 17,21-Dihydroxy-16 β -methylpregna-1,4-diene-3,11,20-trione [1247-42-3].

DEFINITION

Meprednisone contains NLT 97.5% and NMT 102.5% of meprednisone ($C_{22}H_{28}O_5$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B. ULTRAVIOLET ABSORPTION (197U)**
 Analytical wavelength: 238 nm
 Standard solution: 10 μ g/mL of USP Meprednisone RS in methanol
 Sample solution: 10 μ g/mL in methanol
 Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.
- **C. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**
 Diluent: Toluene and alcohol (1:1)
 Sample solution: 20 mg/mL in Diluent
 Chromatographic system
 Spray reagent: 10% (v/v) sulfuric acid in alcohol
 Analysis: Proceed as directed in the chapter. Locate the spots on the plate by spraying with *Spray reagent* and heating at 105° for 10 min.
 Acceptance criteria: Meets the requirements

ASSAY

PROCEDURE

Diluent: Alcohol and chloroform (1:1)
 Standard solution: Prepare as directed in *Single-Steroid Assay* (511), *Standard Preparation* using USP Meprednisone RS.
 Sample solution: 2 mg/mL of previously dried Meprednisone in Diluent
 Instrumental conditions
 Mode: UV
 Analytical wavelength: Maximum at about 238 nm
 Cell: 1 cm
 Analysis
 Samples: *Standard solution* and *Sample solution*
 Proceed as directed in *Single-Steroid Assay* (511), *Procedure* using a solvent system consisting of chloroform, methanol, and water (180:15:1), through the fourth sentence of the second paragraph. Then centrifuge the tubes for 5 min. Determine the absorbances of the supernatants against a blank.
 Calculate the percentage of meprednisone ($C_{22}H_{28}O_5$) in the portion of Meprednisone taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of USP Meprednisone RS in the *Standard solution* (mg/mL)
 C_U = concentration of the *Sample solution* (mg/mL)
 Acceptance criteria: 97.5%–102.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

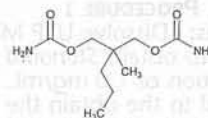
SPECIFIC TESTS

- **LOSS ON DRYING (731)**
 Analysis: Dry at 105° for 3 h.
 Acceptance criteria: NMT 1.0%
- **OPTICAL ROTATION, Specific Rotation (781S)**
 Sample solution: 10 mg/mL in dioxane
 Acceptance criteria: +180° to +188°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS (11)**
 USP Meprednisone RS

Meprobamate



$C_9H_{18}N_2O_4$ 218.25
 1,3-Propanediol, 2-methyl-2-propyl-, dicarbamate;
 2-Methyl-2-propyl-1,3-propanediol dicarbamate [57-53-4].

DEFINITION

Meprobamate contains NLT 97.0% and NMT 101.0% of meprobamate ($C_9H_{18}N_2O_4$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
 Sample: 1 mg in 200 mg
 Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the *Sample*, previously dried, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Meprobamate RS. If a difference appears, dissolve portions of both the *Sample* and the Reference Standard in acetone at a concentration of 8 mg/mL. Dilute 0.1-mL portions of the acetone solutions with 1 mL of *n*-heptane, and remove the solvents by evaporation under nitrogen at a temperature of 30°. Dry the residues under vacuum at room temperature for 30 min, and repeat the test on the residues.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Mobile phase: Acetonitrile and water (30:70)
 Standard solution: 5 mg/mL of USP Meprobamate RS prepared as follows. Dissolve the Standard first in acetonitrile using 30% of final volume. Sonicate if necessary to dissolve, and cool to room temperature. Dilute with water to volume.
 Sample solution: 5 mg/mL of Meprobamate prepared as follows. Dissolve the sample first in acetonitrile using 30% of final volume. Sonicate if necessary to dissolve, and cool to room temperature. Dilute with water to volume.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 25-cm; 4-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: 2 times the retention time of meprobamate

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of meprobamate (C₉H₁₈N₂O₄) in the portion of Meprobamate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Meprobamate RS in the *Standard solution* (mg/mL) C_U = concentration of Meprobamate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–101.0% on the dried basis

IMPURITIES• **ORGANIC IMPURITIES: PROCEDURE 1**

Standard solutions: Dissolve USP Meprobamate RS in alcohol, and mix to obtain *Standard solution A* with a known concentration of 1.0 mg/mL. Dilute quantitatively with alcohol to obtain the *Standard solutions* with the compositions given in *Table 1*.

Table 1

Standard Solution	Dilution	Concentration (mg RS/mL)	Percentage (% for Comparison with Sample)
A	(Undiluted)	1.0	1.0
B	(4 in 5)	0.8	0.8
C	(3 in 5)	0.6	0.6
D	(2 in 5)	0.4	0.4
E	(1 in 5)	0.2	0.2

Sample solution: 100 mg/mL of Meprobamate in alcohol

Chromatographic system(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: Thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel

Application volume: 2 μL

Developing solvent system: Hexane, acetone, and pyridine (70:30:10)

Spray reagent: 5 mg/mL of vanillin in a cooled mixture of sulfuric acid and alcohol (80:20)

AnalysisSamples: *Standard solutions* and *Sample solution*

Position the plate in a chromatographic chamber, and develop the chromatograms in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and air-dry the plate for 15 min. Heat the plate at 100° for 15 min, cool, and spray with *Spray reagent*. Heat the plate at 110° for 15–20 min, cool, and allow the plate to develop blue-purple spots at room temperature. [NOTE—Color development requires about 30–60 min.] Examine the plate, and compare the in-

tensities of any secondary spots of the *Sample solution* with those of the principal spots of the *Standard solutions*.

Acceptance criteria: No secondary spot of the *Sample solution* is larger or more intense than the principal spot of *Standard solution A* (1.0%), and the sum of the intensities of all secondary spots of the *Sample solution* corresponds to NMT 2.0%.

• **ORGANIC IMPURITIES, PROCEDURE 2: LIMIT OF METHYL CARBAMATE****Standard solution:** 1.0 mg/mL of methyl carbamate

Sample solution: Transfer 1.0 g of finely powdered Meprobamate to a beaker, add 5.0 mL of water, and stir to wet the powder completely. Filter the slurry through a small plug of glass wool in the stem of a glass funnel. Use the clear filtrate.

Mobile phase: Water

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 3.9–4.6-mm × 25–30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 50 μL

System suitabilitySample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*

Acceptance criteria: The peak response of the *Sample solution* is not greater than that of the *Standard solution*, corresponding to NMT 0.5% of methyl carbamate.

SPECIFIC TESTS• **LOSS ON DRYING (731)**

Analysis: Dry a sample under vacuum at 60° for 3 h.

Acceptance criteria: NMT 0.5%

• **MELTING RANGE OR TEMPERATURE (741):** 103°–107°, but the range between the beginning and end of melting is NMT 2°.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight containers.• **USP REFERENCE STANDARDS (11)**

USP Meprobamate RS

Meprobamate Oral Suspension**DEFINITION**

Meprobamate Oral Suspension contains NLT 95.0% and NMT 110.0% of the labeled amount of meprobamate (C₉H₁₈N₂O₄).

IDENTIFICATION• **A.****Sample solution:** 2 mL of Oral Suspension

Analysis: Mix the *Sample solution* with 2 mL of acetone and 2 mL of furfural in glacial acetic acid (1 in 100), add 5 mL of hydrochloric acid, and shake.

Acceptance criteria: A purple color is produced, and, on standing, it changes to blue, then to blue-black, and finally to black-brown.

ASSAY• **PROCEDURE**

Sample solution: Transfer an equivalent to 400 mg of meprobamate from Oral Suspension to a separator, and completely extract the meprobamate with 20-mL portions of chloroform, filtering the extracts through a pledget of cotton enclosed in glass wool that previously has been moistened with chloroform. Collect the filtrate

in a conical flask, add several glass beads to the flask, and evaporate on a steam bath to dryness. To the residue add 20 mL of water, heat on a steam bath for several min, then add 40 mL of hydrochloric acid, and reflux for 90 min. Remove the condenser, and continue boiling until the volume is reduced to about 20 mL. Cool to room temperature, add 50 mL of water, and cool in an ice bath. Add 1 drop of methyl red TS, and, while cooling continuously, cautiously neutralize with sodium hydroxide solution (4 in 10) until the indicator begins to change color. Add hydrochloric acid, if necessary, to restore the pink color, then carefully neutralize with 0.1 N sodium hydroxide VS. Add 30 mL of neutral formaldehyde solution (18% w/w).

Analysis: Titrate with 0.1 N sodium hydroxide VS until the solution becomes yellow. Add 0.2 mL of phenolphthalein TS, and continue the titration with 0.1 N sodium hydroxide VS to a distinct pink color. Perform a blank determination. Each mL of the total volume of 0.1 N sodium hydroxide consumed after the addition of the formaldehyde solution is equivalent to 10.91 mg of meprobamate ($C_9H_{18}N_2O_4$).

Acceptance criteria: 95.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements for oral suspension packaged in single-unit containers
- **DELIVERABLE VOLUME (698):** Meets the requirements for oral suspension packaged in multiple-unit containers

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Meprobamate Tablets

DEFINITION

Meprobamate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of meprobamate ($C_9H_{18}N_2O_4$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

Sample: A portion of finely powdered Tablets, equivalent to 800 mg of meprobamate

Analysis: To the *Sample* add 5 mL of dehydrated alcohol, and heat to just below boiling for about 5 min, with occasional swirling. Cool, and filter into 15 mL of solvent hexane. With the aid of suction, filter the crystals that form, and dry at 60°.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion (about 1 mg in 200 mg) from a portion of crystals obtained from the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Meprobamate RS. If a difference appears, dissolve portions of both the *Sample* and the Reference Standard in acetone at a concentration of 8 mg/mL. Dilute 0.1-mL portions of the acetone solutions with 1 mL of *n*-heptane, and remove the solvents by evaporation under nitrogen at a temperature of about 30°. Dry the residues under vacuum at room temperature for 30 min, and repeat the test on the residues.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile and water (30:70)

Phenacetin stock solution: 125 µg/mL of phenacetin in acetonitrile

Phenacetin solution: 25 µg/mL of phenacetin prepared as follows from the *Phenacetin stock solution*. Pipet a suitable volume of *Phenacetin stock solution* into a volumetric flask. Add acetonitrile to fill 30% of the final flask volume, and dilute with water to volume.

Standard solution: 5 mg/mL of USP Meprobamate RS prepared as follows. Transfer a suitable amount of the Reference Standard to a suitable volumetric flask. Dissolve in 30% of the final flask volume of acetonitrile, and dilute with water to volume.

System suitability solution: 5 mg/mL of USP Meprobamate RS and 5 µg/mL of phenacetin prepared as follows. Dissolve a weighed amount of USP Meprobamate RS, first in acetonitrile, using 20% final volume. Shake to dissolve. Add a suitable volume of *Phenacetin solution*, and dilute with water to volume.

Sample solution: Nominally equivalent to 5 mg/mL of meprobamate prepared as follows. Transfer an amount of meprobamate from a portion of finely powdered Tablets (NLT 20) to a suitable volumetric flask. Add acetonitrile to fill 30% of final volume, and shake to dissolve. Dilute with water to volume, and filter, discarding the first 10 mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 3.9–4.6-mm × 25–30-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for meprobamate and phenacetin are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the meprobamate and the phenacetin peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of meprobamate ($C_9H_{18}N_2O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of meprobamate from the *Sample solution*

r_S = peak response of meprobamate from the *Standard solution*

C_S = concentration of USP Meprobamate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of meprobamate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Procedure for a pooled sample

Medium: Deaerated water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Calculate the percentage of the labeled amount of meprobamate ($C_9H_{18}N_2O_4$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

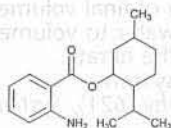
r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

- C_s = concentration of USP Meprobamate RS in the Standard solution (mg/mL)
 V = volume of the Medium, 900 mL
 L = label claim (mg/Tablet)
 Acceptance criteria: NLT 75% (Q) of the labeled amount of meprobamate ($C_9H_{18}N_2O_4$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
USP Meprobamate RS

Meradimate

$C_{17}H_{25}NO_2$ 275.39
 Cyclohexanol, 5-methyl-2-(1-methylethyl)-, 2-aminobenzoate;
 Anthranilic acid, *p*-menth-3-yl ester [134-09-8].

DEFINITION

Meradimate contains NLT 95.0% and NMT 105.0% of meradimate ($C_{17}H_{25}NO_2$).

IDENTIFICATION

- **A. INFRARED ABSORPTION (177F)**
- **B. ULTRAVIOLET ABSORPTION (197U)**
Sample solution: 5 µg/mL in alcohol
Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

ASSAY• **PROCEDURE**

Standard solution: 20.0 mg/mL of USP Meradimate RS in *tert*-butyl methyl ether

Sample solution: 20 mg/mL of Meradimate in *tert*-butyl methyl ether

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 25-m; coated with a 0.1-µm film of G1

Temperatures

Injector: 240°

Detector: 260°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	8	240	10

Carrier gas: Helium

Flow rate: 6 mL/min

Injection type: Split ratio, 30:1

[NOTE—The split ratio can be modified to optimize performance.]

Injection volume: 1 µL

System suitability

Sample: Standard solution

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of meradimate ($C_{17}H_{25}NO_2$) in the portion of Meradimate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the Sample solution

r_s = peak response from the Standard solution

C_s = concentration of USP Meradimate RS in the Standard solution (mg/mL)

C_u = concentration of the Sample solution (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES• **ORGANIC IMPURITIES**

Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Sample: Sample solution

Calculate the percentage of each impurity in the portion of Meradimate taken:

$$\text{Result} = (r_u/r_t) \times 100$$

r_u = peak response of each impurity

r_t = sum of all the peak responses

Acceptance criteria

Any individual impurity: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS• **ACIDITY**

Sample: 5.0 mL of Meradimate

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium hydroxide VS

Endpoint detection: Visual

Analysis: Transfer 50 mL of alcohol to a suitable container, add 1 mL of phenolphthalein TS, and add sufficient volume of Titrant to obtain a persistent pink color. Transfer 50 mL of this solution to a suitable container, add the Sample, and titrate with Titrant.

Acceptance criteria: NMT 0.2 mL of Titrant per mL of Meradimate is necessary.

• **OPTICAL ROTATION, Specific Rotation (781S):** -4° to $+4^\circ$

Sample solution: 10 mg/mL in alcohol

• **REFRACTIVE INDEX (831):** 1.540–1.544 at 20°**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Meradimate RS

Mercaptopurine

$C_5H_4N_4S \cdot H_2O$

170.19

$C_5H_4N_4S$

152.18

6H-Purine-6-thione, 1,7-dihydro-, monohydrate;
 Purine-6-thiol monohydrate [6112-76-1].

Anhydrous [50-44-2].

DEFINITION

Mercaptopurine contains NLT 97.0% and NMT 102.0% of mercaptopurine ($C_5H_4N_4S$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: 0.77 g/L of ammonium acetate in water
Mobile phase: Methanol and *Solution A* (25:75)

Standard stock solution: 0.2 mg/mL of USP Mercaptopurine RS in a mixture of methanol and water (1:1). Transfer USP Mercaptopurine RS into a suitable volumetric flask, and add methanol equivalent to 50% of the final volume. Shake mechanically to dissolve, and dilute with water to volume.

Standard solution: 0.02 mg/mL of USP Mercaptopurine RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: Transfer 25 mg of Mercaptopurine into a 50-mL volumetric flask. Add 25 mL of methanol, shake mechanically for at least 45 min, and dilute with water to volume. Transfer 20 mL of this solution into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: 0.02 mg/mL of Mercaptopurine in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 325 nm

Column: 4.6-mm × 15-cm; 5-μm packing L68

Flow rate: 1.0 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mercaptopurine ($C_5H_4N_4S$) in the portion of Mercaptopurine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mercaptopurine RS in the *Standard solution* (mg/mL)

C_U = concentration of Mercaptopurine in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

• **ORGANIC IMPURITIES**

Solution A: 0.1% (v/v) Formic acid in water

Solution B: Methanol and *Solution A* (2:98)

Solution C: Methanol and *Solution A* (1:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
8	100	0

Table 1 (Continued)

Time (min)	Solution B (%)	Solution C (%)
20	0	100
25	0	100
27	100	0
30	100	0

Standard stock solution: 0.06 mg/mL of USP Mercaptopurine RS in *Solution A*. [NOTE—Use methanol equivalent to 2.5% of the final volume to help dissolve.]

Standard solution: 1.2 μg/mL of USP Mercaptopurine RS in *Solution B* from the *Standard stock solution*

Sensitivity solution: 0.06 μg/mL of USP Mercaptopurine RS in *Solution B* from the *Standard solution*

Sample solution: 0.12 mg/mL of Mercaptopurine in *Solution A*. [NOTE—Inject the *Sample solution* within 1 h of preparation.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 10-cm; 3-μm packing L1

Temperatures

Column: 30°

Sample: 4°

Flow rate: 1.0 mL/min

Injection volume: 50 μL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Tailing factor: NMT 2.0, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Mercaptopurine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of mercaptopurine from the *Standard solution*

C_S = concentration of USP Mercaptopurine RS in the *Standard solution* (mg/mL)

C_U = concentration of Mercaptopurine in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Didanosine related compound A ^a	0.54	6.3	0.15
Mercaptopurine	1.00	—	—
Mercaptopurine disulfide ^b	2.90	4.4	0.15

^a Hypoxanthine.

^b 1,2-Di(9H-purin-6-yl)disulfane.

Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

^a Hypoxanthine.

^b 1,2-Di(9H-purin-6-yl)disulfane.

SPECIFIC TESTS

• PHOSPHORUS

Standard phosphate solution: 43.96 µg/mL of dried monobasic potassium phosphate (equivalent to 10 µg of phosphorus)

Standard solution: Transfer 2 mL of *Standard phosphate solution* to a 25-mL volumetric flask. Add 1 mL of 15 N sulfuric acid, 0.5 mL of nitric acid, 0.75 mL of ammonium molybdate TS, and 1 mL of aminonaphtholsulfonic acid TS, then dilute with water to volume, and mix. Allow to stand for 5 min.

Sample solution: Digest 200 mg with 2 mL of 15 N sulfuric acid in a large test tube, periodically adding nitric acid, dropwise and with caution. Continue heating until practically all of the liquid has evaporated and the residue is colorless. Transfer the residue, with the aid of small portions of water, to a 25-mL volumetric flask. Add 1 mL of 15 N sulfuric acid, 0.5 mL of nitric acid, 0.75 mL of ammonium molybdate TS, and 1 mL of aminonaphtholsulfonic acid TS, then dilute with water to volume. Allow to stand for 5 min.

Blank: Transfer 2 mL of 15 N sulfuric acid to a large test tube, periodically adding nitric acid, dropwise and with caution. Continue heating until practically all of the liquid has evaporated and the residue is colorless. Transfer the residue, with the aid of small portions of water, to a 25-mL volumetric flask. Add 1 mL of 15 N sulfuric acid, 0.5 mL of nitric acid, 0.75 mL of ammonium molybdate TS, and 1 mL of aminonaphtholsulfonic acid TS, then dilute with water to volume. Allow to stand for 5 min.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV-Vis

Analytical wavelength: 750 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Acceptance criteria: The absorbance of the *Sample solution* is NMT that of the *Standard solution* (NMT 100 ppm).

• WATER DETERMINATION, Method I (921)

Medium: 30 mL of methanol and 5 g of salicylic acid in the titration vessel

Sample: 0.3 g of Mercaptopurine

Acceptance criteria: NMT 12.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Mercaptopurine RS

Mercaptopurine Tablets

DEFINITION

Mercaptopurine Tablets contain NLT 93.0% and NMT 110.0% of the labeled amount of mercaptopurine ($C_5H_4N_4S \cdot H_2O$).

IDENTIFICATION

• **A.** The UV absorption spectrum exhibits a maximum at 325 ± 2 nm, and the ratio A_{255}/A_{325} does not exceed 0.09.

Sample: 5 µg/mL of mercaptopurine in a mixture of methanol and water (1:1), from the *Sample solution* in the Assay

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: 0.77 g/L of ammonium acetate in water

Solution B: Methanol and *Solution A* (5:95)

Solution C: Methanol and *Solution A* (30:70)

Mobile phase: *Solution B* and *Solution C* (80:20)

Diluent: Methanol and water (1:1)

Standard solution: 0.25 mg/mL of USP Mercaptopurine RS in a mixture of methanol and water (1:1). Transfer USP Mercaptopurine RS into a suitable volumetric flask, and add methanol equivalent to 50% of the final volume. Shake mechanically to dissolve, and dilute with water to volume.

Sample stock solution: 0.5 mg/mL of mercaptopurine in *Diluent* from NLT 5 Tablets. Place the Tablets into a suitable volumetric flask, add methanol equivalent to 50% of the final volume, and shake mechanically for a minimum of 30 min. Dilute with water to volume. Pass through a PVDF filter of 0.45-µm pore size, and discard the first 3 mL of filtrate.

Sample solution: 0.25 mg/mL of mercaptopurine in *Diluent* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mercaptopurine ($C_5H_4N_4S \cdot H_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mercaptopurine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mercaptopurine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of mercaptopurine, 170.19

M_{r2} = molecular weight of anhydrous mercaptopurine, 152.18

Acceptance criteria: 93.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Mobile phase: 0.1% acetic acid in water

Standard solution: USP Mercaptopurine RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* to a

concentration that is similar to the *Standard solution*, if necessary.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 3.9-mm × 15-cm; packing L1

Flow rate: 2.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for mercaptopurine is NLT 4 min.]

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 80% (Q) of the labeled amount of $C_5H_4N_4S \cdot H_2O$ is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Apparatus 2, Chromatographic system, and

Analysis: Proceed as directed for *Test 1*.

Time: 120 min

Tolerances: NLT 80% (Q) of the labeled amount of $C_5H_4N_4S \cdot H_2O$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

IMPURITIES

Organic Impurities

• PROCEDURE

Solution A: 0.1% (v/v) formic acid in water

Solution B: Methanol and *Solution A* (2:98)

Solution C: Methanol and *Solution A* (1:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
8	100	0
20	0	100
25	0	100
27	100	0
30	100	0

Standard stock solution: 0.06 mg/mL of USP Mercaptopurine RS in *Solution A*. [NOTE—Use methanol equivalent to 2.5% of the final volume to help dissolve.]

Standard solution: 1.2 µg/mL of USP Mercaptopurine RS in *Solution B* from the *Standard stock solution*

Sensitivity solution: 0.06 µg/mL of USP Mercaptopurine RS in *Solution B* from the *Standard solution*

Sample stock solution: 0.5 mg/mL of mercaptopurine in a mixture of methanol and *Solution A* (1:9) from NLT 5 Tablets. Place the Tablets into a suitable volumetric flask, add methanol equivalent to 10% of the final volume, and shake mechanically for a minimum of 30 min. Dilute with *Solution A* to volume. Pass through a PVDF filter of 0.45-µm pore size, and discard the first 3 mL of filtrate.

Sample solution: 0.12 mg/mL of mercaptopurine in *Solution A*. Transfer 6.0 mL of the *Sample stock solution* into a 25-mL volumetric flask, and dilute with *Solution A* to volume. Pass through a PVDF filter of 0.45-µm pore size, and discard the first 5 mL of filtrate. [NOTE—Inject the *Sample solution* within 1 h of preparation.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Temperature

Column: 30°

Sample: 4°

Flow rate: 1.0 mL/min

Injection size: 50 µL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Tailing factor: NMT 2.0, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each impurity from the *Sample solution*

r_s = peak response of mercaptopurine from the *Standard solution*

C_s = concentration of USP Mercaptopurine RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of mercaptopurine in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria

Individual impurities: See *Table 2*. [NOTE—Disregard any impurity peak less than 0.05%.]

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Didanosine related compound A ^a	0.54	6.3	0.3
Mercaptopurine	1.00	—	—
Mercaptopurine disulfide ^b	2.90	4.4	0.4
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	0.6

^a Hypoxanthine.

^b 1,2-Di(9H-purin-6-yl)disulfane.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

- **USP REFERENCE STANDARDS (11)**
USP Mercaptopurine RS

Ammoniated Mercury

Hg(NH₂)Cl 252.07
Mercury amide chloride [10124-48-8].

DEFINITION

Ammoniated Mercury contains NLT 98.0% and NMT 100.5% of ammoniated mercury $[\text{Hg}(\text{NH}_2)\text{Cl}]$.

IDENTIFICATION

- **A.**
Sample: 0.1 g
Analysis: Place the *Sample* in a cold solution of 1 g of sodium thiosulfate in 2 mL of water.
Acceptance criteria: The *Sample* is soluble, with the evolution of ammonia. When this solution is heated gently, a rust-colored mixture is formed, from which a red precipitate is obtained on centrifugation. If the solution is strongly heated, a black mixture forms.
- **B.**
Sample: A suitable quantity
Analysis: Heat the *Sample* with 1 N sodium hydroxide.
Acceptance criteria: The solution becomes yellow, and ammonia is evolved.
- **C.**
Sample solution: A suitable quantity in warm acetic acid
Analysis: *Sample solution* with potassium iodide TS
Acceptance criteria: The solution yields a red precipitate that is soluble in an excess of the reagent. The solution yields a white precipitate with silver nitrate TS.

ASSAY**• PROCEDURE**

Sample solution: Mix 0.25 g of Ammoniated Mercury with 10 mL of water. Add 3 g of potassium iodide, mix occasionally until dissolved, add about 40 mL of water, and add methyl red TS.

Analysis: Titrate with 0.1 N hydrochloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N hydrochloric acid is equivalent to 12.60 mg of ammoniated mercury $[\text{Hg}(\text{NH}_2)\text{Cl}]$.

Acceptance criteria: 98.0%–100.5%

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.2%

• MERCUROS COMPOUNDS

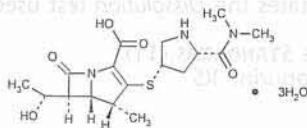
Sample: 2.5 g

Analysis: Dissolve the *Sample* in 25 mL of warm hydrochloric acid. Pass through a tared filtering crucible, wash with water, and dry at 60° to constant weight.

Acceptance criteria: NMT 0.2%; the weight of the residue does not exceed 5 mg.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

Meropenem

$\text{C}_{17}\text{H}_{25}\text{N}_3\text{O}_5\text{S} \cdot 3\text{H}_2\text{O}$ 437.51

1-Azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, 3-[[5-[(dimethylamino)carbonyl]-3-pyrrolidinyl]thio]-6-(1-hydroxyethyl)-4-methyl-7-oxo, trihydrate, [4R-[3(3S*, 5S*), 4α, 5β, 6β(R*)]]-, (4R, 5S, 6S)-3-[[[(3S, 5S)-5-(Dimethylcarbamoyl)-3-pyrrolidinyl]thio]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-carboxylic acid, trihydrate [119478-56-7].

Anhydrous 383.47 [96036-03-2].

» Meropenem contains not less than 98.0 percent and not more than 101.0 percent of $\text{C}_{17}\text{H}_{25}\text{N}_3\text{O}_5\text{S}$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers. Store the dry powder at controlled room temperature.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards (11)—

USP Endotoxin RS

USP Meropenem RS

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 30 µg per mL.

Medium: water.

Specific rotation (781): between −17° and −21°, measured at 20°.

Test solution: 5 mg per mL, in water.

pH (791): between 4.0 and 6.0, in a solution (1 in 100).

Water Determination, Method 1c (921): between 11.4% and 13.4%.

Residue on ignition (281): not more than 0.1%, igniting at $500 \pm 50^\circ$, instead of at $800 \pm 25^\circ$. Use a desiccator containing silica gel.

Delete the following:**• Heavy metals—**

Sodium sulfide reagent—Dissolve 5 g of sodium sulfide in a mixture of 10 mL of water and 30 mL of glycerin. Preserve in well-filled, light-resistant bottles, and use within 3 months.

Test solution—Transfer 1.0 g of Meropenem to a quartz or porcelain crucible, cover loosely with a lid, and carbonize by gentle ignition. After cooling, add 2 mL of nitric acid and 5 drops of sulfuric acid, heat cautiously until white fumes evolve, and incinerate by ignition at 500° to 600°. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. Moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Add 1 drop of phenolphthalein TS, add ammonia TS, dropwise, until the solution develops a pale red color, and add 2 mL of 1 N acetic acid. Filter, if necessary, to obtain a clear solution, washing the filter with 10 mL of water. Transfer the filtrate and the washing to a 50-mL color-comparison tube, and add water to obtain a volume of 50 mL.

Standard solution—Evaporate a mixture of 2 mL of nitric acid, 5 drops of sulfuric acid, and 2 mL of hydrochloric acid on a water bath, further evaporate to dryness on a hot sand bath, and moisten the residue with 3 drops of hydrochloric acid. Proceed as directed for *Test solution*, beginning with "add 10 mL of hot water," except add water to obtain a volume of 49 mL. Add 1.0 mL of *Standard Lead Solution* (see *Heavy Metals* (231)).

Procedure—To the tubes containing the *Test solution* and the *Standard solution*, add 1 drop of *Sodium sulfide reagent*, mix, and allow to stand for 5 minutes. The color in the tube containing the *Test solution* is not darker than the color in the tube containing the *Standard solution* (0.001%). (Official 1-Jan-2018)

Limit of acetone—

Internal standard solution—Prepare a solution in dimethylformamide containing 0.05 µL of ethyl acetate per mL.

Standard solution—Transfer about 50 mg of acetone, accurately weighed, to a 100-mL volumetric flask, dilute with dimethylformamide to volume, and mix. To 1.0 mL of this

solution, add 10.0 mL of the *Internal standard solution*, and mix.

Test solution—Dissolve 100 mg of Meropenem, accurately weighed, in 0.2 mL of dimethylformamide and 2.0 mL of *Internal standard solution*.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 3-mm × 2-m column that contains support S2 and is maintained at a constant temperature of about 150°. The injection port temperature is maintained at about 170°. Nitrogen is the carrier gas, with the flow rate adjusted so that the retention time for acetone is about 3 minutes.

Procedure—Separately inject equal volumes (about 2 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for the acetone peak and the internal standard peak. Calculate the percentage of acetone in the portion of Meropenem taken by the formula:

$$(W_A/5W_U)(R_U/R_S)$$

in which W_A is the weight, in mg, of acetone in the *Standard solution*; W_U is the quantity, in mg, of Meropenem in the *Test solution*; and R_U and R_S are the peak area ratios of acetone to the internal standard obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 0.05% is found.

Chromatographic purity—

Diluted phosphoric acid—Dilute 10 mL of phosphoric acid with water to make 100 mL of solution.

Solvent—Transfer 1.0 mL of triethylamine to a 1000-mL volumetric flask containing 900 mL of water. Adjust with *Diluted phosphoric acid* to a pH of 5.0 ± 0.1 , dilute with water to volume, and mix.

Mobile phase—Transfer 1.0 mL of triethylamine to a 1000-mL volumetric flask containing 900 mL of water. Adjust with *Diluted phosphoric acid* to a pH of 5.0 ± 0.1 , dilute with water to volume, and mix. Mix this solution with 70 mL of acetonitrile. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard solution—Prepare a solution of USP Meropenem RS in *Solvent* having a known concentration of about 0.025 mg of USP Meropenem RS per mL. [NOTE—Immediately after preparation, store this solution in a refrigerator and use within 24 hours.]

Test solution—Dissolve an accurately weighed quantity of Meropenem quantitatively in *Solvent* to obtain a solution having a known concentration of about 5 mg per mL. Use this *Test solution* immediately.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 1.6 mL per minute, and is adjusted so that the retention time of meropenem is between 5 and 7 minutes. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, using a period of chromatography for the *Test solution* that is about 3 times the retention time of meropenem, and measure the peak responses. Major impurity peaks may be observed at retention times of about 0.45 and 1.9 in relation to the retention time of meropenem. Calculate the percentage of each im-

purity in the chromatogram obtained from the *Test solution* by the formula:

$$(C_S/C_U)(P)(r_i/r_s)$$

in which C_S is the concentration, in mg per mL, of USP Meropenem RS in the *Standard solution*; C_U is the concentration, in mg per mL, of Meropenem in the *Test solution*; P is the stated percentage, calculated on the anhydrous basis, of meropenem in USP Meropenem RS; r_i is the peak response of any individual impurity obtained from the *Test solution*; and r_s is the peak response of meropenem obtained from the *Standard solution*. Not more than 0.3% of any of two major impurities is found, calculated on the anhydrous basis; not more than 0.1% of any other impurity is found, calculated on the anhydrous basis; and the sum of all such other impurities is not more than 0.3%.

Other requirements—Where the label states that Meropenem is sterile, it meets the requirements for *Sterility Tests* (71) and for *Bacterial endotoxins* under *Meropenem for Injection*. Where the label states that Meropenem must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Meropenem for Injection*.

Assay—

Diluted phosphoric acid—Dilute 10 mL of phosphoric acid with water to make 100 mL of solution.

Solvent—Transfer 1.0 mL of triethylamine to a 1000-mL volumetric flask containing 900 mL of water. Adjust with *Diluted phosphoric acid* to a pH of 5.0 ± 0.1 , dilute with water to volume, and mix.

Mobile phase—Prepare a mixture of *Solvent* and methanol (5:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Transfer about 25 mg of USP Meropenem RS, accurately weighed, to a 50-mL volumetric flask, add *Solvent*, swirl to dissolve, dilute with *Solvent* to volume, and mix. [NOTE—Immediately after preparation, store this solution in a refrigerator. It may be used for 24 hours.]

Assay preparation—Transfer about 25 mg of Meropenem, accurately weighed, to a 50-mL volumetric flask, add *Solvent*, swirl to dissolve, dilute with *Solvent* to volume, and mix. Use this solution immediately after preparation.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 300-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. Adjust the flow rate so that the retention time for meropenem is about 6 to 8 minutes. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 5 µL) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{17}H_{25}N_3O_5S$ in the portion of Meropenem taken by the formula:

$$(W_S/W_U)(P)(r_U/r_S)$$

in which W_S is the weight, in mg, of USP Meropenem RS taken to prepare the *Standard preparation*, calculated on the anhydrous basis; W_U is the weight, in mg, of Meropenem taken to prepare the *Assay preparation*; P is the stated percentage, calculated on the anhydrous basis, of meropenem in USP Meropenem RS; and r_U and r_S are the meropenem peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Meropenem for Injection

DEFINITION

Meropenem for Injection is a sterile dry mixture of Meropenem and Sodium Carbonate. It contains NLT 90.0% and NMT 120.0% of the labeled amount of meropenem ($C_{17}H_{25}N_3O_5S$).

IDENTIFICATION

- A.** The retention time of the meropenem peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Solution A: 1:10 solution of phosphoric acid and water

Buffer: Dilute 15 mL of tetrabutylammonium hydroxide solution (25% in water) with water to 750 mL. Adjust with *Solution A* to a pH of 7.5 ± 0.1 .

Mobile phase: Acetonitrile, methanol, and *Buffer* (150:100:750)

Standard solution: 0.11 mg/mL of USP Meropenem RS in *Mobile phase*. Immediately after preparation, store this solution in a refrigerator, and use within 24 h.

Sample stock solution 1 (where it is represented as being a single-dose container): Nominally 1 mg/mL of meropenem, prepared as follows. Constitute a container of Meropenem for Injection with a volume of water, corresponding to the quantity of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and transfer to a suitable volumetric flask. Dilute with water to volume, and mix.

Sample solution 1: Nominally 0.1 mg/mL of meropenem in *Mobile phase* from *Sample stock solution 1*. Hold this *Sample solution 1* for 2 h at $25 \pm 1^\circ$ before testing.

Sample stock solution 2 (where the label states the quantity of meropenem in a given volume of constituted solution): Nominally 1 mg/mL of meropenem, prepared as follows. Constitute a container of Meropenem for Injection with a volume of water corresponding to the quantity of solvent specified in the labeling, and dilute with water.

Sample solution 2: Nominally 0.1 mg/mL of meropenem in *Mobile phase* from *Sample stock solution 2*. Hold this *Sample solution 2* for 2 h at $25 \pm 1^\circ$ before testing.

Chromatographic system

(See *Chromatography* (621), *System Suitability*)

Mode: LC

Detector: UV 300 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1.5 mL/min. [NOTE—Adjust the flow rate to obtain a retention time for meropenem of about 6–8 min.]

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Tailing factor: NMT 1.5

Analysis

Samples: *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of the labeled amount of meropenem ($C_{17}H_{25}N_3O_5S$) in the portion of Meropenem for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of meropenem from *Sample solution 1* or *Sample solution 2*

r_S = peak response of meropenem from the *Standard solution*

C_S = concentration of USP Meropenem RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of meropenem in *Sample solution 1* or *Sample solution 2* (mg/mL)

P = potency of meropenem in USP Meropenem RS (mg/mg)

Acceptance criteria: 90.0%–120.0%

OTHER COMPONENTS

CONTENT OF SODIUM

Solution A: 38.1 g/L of potassium chloride in water

Standard stock solution: 25.42 μ g/mL of sodium chloride (previously dried at 105° for 2 h) in water

Standard solution: 2.5 μ g/mL of sodium chloride from the *Standard stock solution* mixed first with *Solution A* to 10% of the final volume and diluted with water to volume

Sample stock solution 1 (where it is represented as being a single-dose container): Nominally 0.125 mg/mL of meropenem prepared as follows. Constitute a container of Meropenem for Injection with a volume of water corresponding to the quantity of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and transfer to a suitable volumetric flask. Dilute with water to volume.

Sample stock solution 2 (where the label states the quantity of meropenem in a given volume of constituted solution): Nominally 0.125 mg/mL of meropenem prepared as follows. Constitute a container of Meropenem for Injection with a volume of water, corresponding to the quantity of solvent specified in the labeling. Transfer the constituted solution to a suitable volumetric flask, and dilute with water to volume.

Sample solution: Nominally 0.0125 mg/mL of meropenem from *Sample stock solution 1* or *Sample stock solution 2* mixed first with *Solution A* to 10% of the final volume, and dilute with water to volume

Blank: 1:10 mixture of *Solution A* and water

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 589.6 nm sodium emission line

Burner: Single-slot

Flame: Air–acetylene

Lamp: Sodium hollow-cathode

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of sodium (Na) in the portion of Meropenem for Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of sodium chloride in the *Standard solution* (μ g/mL)

C_U = nominal concentration of meropenem in the *Sample solution* (μ g/mL)

M_{r1} = atomic weight of sodium, 22.99

M_{r2} = molecular weight of sodium chloride, 58.44

Acceptance criteria: 80%–120% of the labeled amount of sodium

PERFORMANCE TESTS

- UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

IMPURITIES**• ORGANIC IMPURITIES**

Solution A: 1:10 solution of phosphoric acid and water
Buffer: Mix 1 mL of triethylamine and 900 mL of water. Adjust with *Solution A* to a pH of 5.0 ± 0.1 , and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (70:1000)

Standard solution: 0.029 mg/mL of USP Meropenem RS in *Buffer*. Store this solution in a refrigerator immediately after preparation, and use within 24 h.

Sample solution: Nominally prepare 5 mg/mL of meropenem in *Buffer* from Meropenem for Injection. This solution has to be prepared fresh and used immediately.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Temperature: 40°

Flow rate: 1.6 mL/min. [NOTE—Adjust to obtain a retention time for meropenem of 5–7 min.]

Injection volume: 10 μ L

Run time: 3 times the retention time of meropenem

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Tailing factor: NMT 1.5

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Meropenem for Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times 100$$

r_u = peak response of each individual impurity from the *Sample solution*

r_s = peak response of meropenem from the *Standard solution*

C_s = concentration of USP Meropenem RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of meropenem in the *Sample solution* (mg/mL)

P = potency of meropenem in USP Meropenem RS (mg/mg)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Meropenem impurity I ^a	0.45	0.8
Meropenem impurity II ^a	1.9	0.6

^a Specified, unidentified impurities.

SPECIFIC TESTS

• BACTERIAL ENDOTOXINS TEST (85): NMT 0.125 USP Endotoxin Unit/mg of meropenem

• CONSTITUTED SOLUTION: At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

• LOSS ON DRYING (731)

Analysis: Dry in a vacuum at 65° for 6 h.

Acceptance criteria: 9.0%–12.0%

• PARTICULATE MATTER IN INJECTIONS (788): Meets the requirements for small-volume injections

• pH (791)

Sample solution: 50 mg/mL

Acceptance criteria: 7.3–8.3

• STERILITY TESTS (71): Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*

ADDITIONAL REQUIREMENTS**Change to read:**

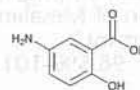
• PACKAGING AND STORAGE: Preserve in tight containers as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017). Store at controlled room temperature.

• LABELING: Meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. Label it to state the quantity, in mg, of sodium (Na) in a given dosage of meropenem.

• USP REFERENCE STANDARDS (11)

USP Endotoxin RS

USP Meropenem RS

Mesalamine

$C_7H_7NO_3$

Benzoic acid, 5-amino-2-hydroxy-;

5-Aminosalicylic acid [89-57-6].

153.14

DEFINITION

Mesalamine contains NLT 98.5% and NMT 101.5% of mesalamine ($C_7H_7NO_3$), calculated on the dried basis.

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

• B. The retention time of the mesalamine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Buffer: Transfer 7.1 g of anhydrous dibasic sodium phosphate and 6.9 g of monobasic sodium phosphate to a 1000-mL volumetric flask, add 500 mL of water, and swirl to dissolve. Add 7.5 mL of a solution of tetrabutylammonium hydroxide 30-hydrate in methanol (1 in 4), dilute with water to volume, and mix.

Mobile phase: Methanol and *Buffer* (15:85)

System suitability solution: 0.25 mg/mL of 4-aminosalicylic acid and 0.4 mg/mL of USP Mesalamine RS in *Mobile phase*

Standard stock solution: 1 mg/mL of USP Mesalamine RS in *Mobile phase*

Standard solution: 0.4 mg/mL of USP Mesalamine RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: 1 mg/mL of Mesalamine in *Mobile phase*

Sample solution: 0.4 mg/mL of Mesalamine in *Mobile phase* from the *Sample stock solution*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4-mm × 30-cm; 10-μm packing L1**Flow rate:** 2 mL/min**Injection volume:** 15 μL**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 2.0 between 4-aminosalicylic acid and mesalamine, *System suitability solution***Tailing factor:** NMT 2.5, *Standard solution***Relative standard deviation:** NMT 0.73%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of mesalamine (C₇H₇NO₃) in the portion of Mesalamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of mesalamine from the *Sample solution* r_S = peak response of mesalamine from the *Standard solution* C_S = concentration of USP Mesalamine RS in the *Standard solution* (mg/mL) C_U = concentration of Mesalamine in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.5%–101.5% on the dried basis**IMPURITIES**• **CHLORIDE AND SULFATE, Chloride** (221)**Sample solution:** Disperse 500 mg in 40 mL of water, sonicate for 5 min, and filter the dispersion. Use the filtrate.**Analysis:** Add 1 mL of nitric acid to the *Sample solution*.**Acceptance criteria:** No more chloride than corresponds to 0.7 mL of 0.020 N hydrochloric acid (0.1%)• **CHLORIDE AND SULFATE, Sulfate** (221)**Sample solution:** Dissolve 500 mg in water. Filter if necessary. Use the filtrate.**Acceptance criteria:** The *Sample solution* shows no more sulfate than corresponds to 1.0 mL of 0.02 N sulfuric acid (0.2%).**Delete the following:**• **HEAVY METALS, Method II** (231): NMT 0.002% (Official 1, Jan-2018)• **RESIDUE ON IGNITION** (281): NMT 0.2%• **HYDROGEN SULFIDE AND SULFUR DIOXIDE****Analysis:** Dissolve about 500 mg in 5 mL of 1 N sodium hydroxide, add 6 mL of 3 N hydrochloric acid, and stir vigorously. Hold a piece of moistened lead acetate test paper over the mixture.**Acceptance criteria:** The test paper so obtained does not become discolored.• **CONTENT OF 3-AMINOSALICYLIC ACID AND OTHER RELATED IMPURITIES**

[NOTE—Use this test to measure 3-aminosalicylic acid and other related impurities not measured in the test for

Content of Aniline, 2-Aminophenol, and 4-Aminophenol.]**Mobile phase:** Dissolve 1.36 g of monobasic potassium phosphate and 2.2 g of sodium 1-octanesulfonate in 890 mL of water, and adjust with phosphoric acid to a pH of 2.2. Pass through a filter of 0.5-μm or finer pore size. To the filtrate add 80 mL of methanol and 30 mL of acetonitrile.**Standard solution:** 1 μg/mL each of USP Mesalamine RS and 3-aminosalicylic acid in *Mobile phase***Sample solution:** 0.5 mg/mL of Mesalamine in *Mobile phase*. Initially add about 75% of the final volume of *Mobile phase*, and sonicate briefly to dissolve. Dilute with *Mobile phase* to volume, and mix.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 220 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L7**Flow rate:** 1.2 mL/min**Injection volume:** 20 μL**Run time:** 3 times the retention time of mesalamine**System suitability****Sample:** *Standard solution*

[NOTE—See Table 1 for the relative retention times.]

Suitability requirements**Resolution:** NLT 2 between mesalamine and 3-aminosalicylic acid**Relative standard deviation:** NMT 5.0% for both mesalamine and 3-aminosalicylic acid**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 3-aminosalicylic acid:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of 3-aminosalicylic acid from the *Sample solution* r_S = peak response of 3-aminosalicylic acid from the *Standard solution* C_S = concentration of 3-aminosalicylic acid in the *Standard solution* (μg/mL) C_U = concentration of Mesalamine in the *Sample solution* (μg/mL)

Calculate the percentage of any other impurity:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of any individual impurity from the *Sample solution* r_S = peak response of mesalamine from the *Standard solution* C_S = concentration of USP Mesalamine RS in the *Standard solution* (μg/mL) C_U = concentration of Mesalamine in the *Sample solution* (μg/mL)**Acceptance criteria:** See Table 1.**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mesalamine	1.0	—
3-Aminosalicylic acid	1.3	0.2
Any other impurity	—	0.2
Total impurities	—	1.0

• **CONTENT OF ANILINE, 2-AMINOPHENOL, AND 4-AMINOPHENOL**
Standard stock solution: 0.05 mg/mL of aniline, 2 mg/mL of 2-aminophenol, and 2 mg/mL of USP 4-Aminophenol RS in methanol**Standard solution:** 0.5 μg/mL of aniline, 20 μg/mL of 2-aminophenol, and 20 μg/mL of USP 4-Aminophenol RS from the *Standard stock solution* in methylene chloride**Sample solution:** 100 mg/mL of Mesalamine in methylene chloride. Allow to settle, and use the clear methylene chloride solution.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 10-m fused-silica capillary; 2.65- μ m film of G27

Temperatures

Injection port: 280°

Detector: 300°

Column: See *Table 2*.**Table 2**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	—	70	2
70	30	150	1

Carrier gas: Helium

Flow rate: 15 mL/min

Injection volume: 2 μ L**System suitability**Sample: *Standard solution*[NOTE—See *Table 3* for the relative retention times.]**Suitability requirements**

Resolution: NLT 2.0 between aniline and 2-aminophenol; NLT 2.0 between 2-aminophenol and 4-aminophenol

Relative standard deviation: NMT 10.0% for aniline, 2-aminophenol, and 4-aminophenol

AnalysisSamples: *Standard solution* and *Sample solution*

Calculate the percentage of aniline, 2-aminophenol, and 4-aminophenol in the portion of Mesalamine taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

 r_u = peak response of aniline, 2-aminophenol, or 4-aminophenol from the *Sample solution* r_s = peak response of aniline, 2-aminophenol, or 4-aminophenol from the *Standard solution* C_s = concentration of aniline, 2-aminophenol, or USP 4-Aminophenol RS in the *Standard solution* (μ g/mL) C_u = concentration of Mesalamine in the *Sample solution* (μ g/mL)Acceptance criteria: See *Table 3*.**Table 3**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aniline	0.5	0.0005
2-Aminophenol	0.9	0.02
4-Aminophenol	1.0	0.02

SPECIFIC TESTS**• CLARITY OF SOLUTION**

Sample solution: Freshly prepare a solution of 0.25 g of Mesalamine in 10 mL of 1 N hydrochloric acid.

Acceptance criteria: The *Sample solution* is clear.**• LOSS ON DRYING (731)**

Analysis: Dry under vacuum at 105° for 3 h.

Acceptance criteria: NMT 0.5%

• PH (791)

Sample: A suspension (1 in 40)

Acceptance criteria: 3.5–4.5

ADDITIONAL REQUIREMENTS**• PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.**• USP REFERENCE STANDARDS (11)**

USP 4-Aminophenol RS

4-Aminophenol.

 C_6H_7NO 109.13

USP Mesalamine RS

Mesalamine Extended-Release Capsules**DEFINITION**Mesalamine Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of mesalamine ($C_7H_7NO_3$).**IDENTIFICATION****• A. INFRARED ABSORPTION (197K)**

Sample: Use the powdered, undried Capsule contents.

Analysis: Record the spectra in the range between 2000 cm^{-1} and 1240 cm^{-1} .

Acceptance criteria: Meet the requirements

ASSAY**• PROCEDURE****Buffer:** Dissolve 6.8 g of monobasic potassium phosphate and 1.65 g of sodium hydroxide in 800 mL of water. Adjust with 1 N sodium hydroxide to a pH of 7.5, dilute with water to 1000 mL, and mix.**Solution A:** Dissolve 3.4 g of tetrabutylammonium hydrogen sulfate and 1.4 g of sodium acetate trihydrate in 1000 mL of water. Adjust with 1 N sodium hydroxide to a pH of 6.6. Add 200 mL of acetonitrile, mix, and pass through a filter of 0.5- μ m or finer pore size. [NOTE—Increasing the proportion of acetonitrile decreases the retention times. Prepare fresh daily.]**Solution B:** Dissolve 4.6 g of tetrabutylammonium hydrogen sulfate and 1.9 g of sodium acetate trihydrate in 1000 mL of water, and adjust with 1 N sodium hydroxide to a pH of 6.6. Add 650 mL of acetonitrile, mix, and pass through a filter of 0.5- μ m or finer pore size. [NOTE—Prepare fresh daily.]Mobile phase: See *Table 1*.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
7	0	100
15	0	100
17	100	0
20	100	0

Internal standard solution: 35 mg/mL of sodium benzoate in *Buffer***Standard solution:** Transfer about 50 mg of USP Mesalamine RS to a 100-mL volumetric flask. Add 4.0 mL of the *Internal standard solution*, dilute with *Buffer* to volume, and mix. Dilute 5.0 mL of this solution with *Buffer* to 25 mL.

Sample solution: Transfer, as completely as possible, the contents of NLT 20 Capsules to a suitable tared container, and determine the average weight of the contents of a Capsule. Finely powder the Capsule contents so that the powder thus obtained passes through a No. 40 sieve (see *Powder Fineness* (811)). Transfer a portion of the powder, nominally equivalent to about 250 mg of mesalamine, to a 500-mL volumetric flask. Add 20.0 mL of the *Internal standard solution* and about 300 mL of *Buffer*, and shake by mechanical means for 1 h. Dilute with *Buffer* to volume, and mix. Transfer 5.0 mL of this solution to a 25-mL volumetric flask. Dilute with *Buffer* to volume, mix, and pass about 10 mL of this solution through a filter of 0.5- μ m or finer pore size. Use the filtrate as the *Sample solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for mesalamine and sodium benzoate are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between mesalamine and sodium benzoate

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) in the portion of Capsule contents taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of mesalamine to sodium benzoate from the *Sample solution*

R_S = peak response ratio of mesalamine to sodium benzoate from the *Standard solution*

C_S = concentration of USP Mesalamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mesalamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Buffer: 0.05 M pH 7.5 phosphate buffer prepared as follows. Dissolve 6.8 g of monobasic potassium phosphate and 1 g of sodium hydroxide in water to make 1000 mL of solution, and adjust with 10 N sodium hydroxide to a pH of 7.50 ± 0.05 .

Medium: *Buffer*, 900 mL

Apparatus 2: 100 rpm

Times: 1, 2, 4, and 8 h

Standard solution: A known concentration of USP Mesalamine RS in *Medium*

Sample solution: Filter portions of the solution under test suitably diluted with *Medium*, if necessary.

Analysis: Calculate the percentages of the labeled amount of mesalamine ($C_7H_7NO_3$) dissolved at the wavelength of maximum absorbance at about 330 nm by comparing the UV absorbances of the *Sample solution* with that of the *Standard solution*.

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved
1	5%–25%
2	30%–50%

Table 2 (Continued)

Time (h)	Amount Dissolved
4	60%–90%
8	NLT 85%

The percentages of the labeled amount of mesalamine ($C_7H_7NO_3$) dissolved at the times specified conform to *Acceptance Table 2* in (711).

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
USP Mesalamine RS

Mesalamine Rectal Suspension

DEFINITION

Mesalamine Rectal Suspension is a suspension of Mesalamine in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of mesalamine ($C_7H_7NO_3$). It contains one or more suitable preservatives.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer: Transfer 7.1 g of anhydrous dibasic sodium phosphate and 6.9 g of monobasic sodium phosphate to a 1000-mL volumetric flask, add 500 mL of water, and swirl to dissolve. Add 7.5 mL of a solution of tetrabutylammonium hydroxide in methanol (1 in 4), dilute with water to volume, and mix.

Mobile phase: Methanol and *Buffer* (15:85)

System suitability solution: 0.25 mg/mL of 4-aminosalicylic acid and 0.4 mg/mL of USP Mesalamine RS in *Mobile phase*

Standard stock solution: 1 mg/mL of USP Mesalamine RS in *Mobile phase*

Standard solution: 0.4 mg/mL of USP Mesalamine RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Transfer an accurately measured, well-shaken quantity of Rectal Suspension, nominally equivalent to about 100 mg of mesalamine, to a 100-mL volumetric flask. Add 55 mL of *Mobile phase*, and dissolve by shaking for about 10 min. Dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass this solution through a suitable filter of 0.5- μ m or finer pore size, and use the filtrate as the *Sample solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm \times 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 15 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between 4-aminosalicylic acid and mesalamine, *System suitability solution*

Tailing factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) in the portion of Rectal Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesalamine from the *Sample solution*

r_S = peak response of mesalamine from the *Standard solution*

C_S = concentration of USP Mesalamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mesalamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• CONTENT OF SODIUM BENZOATE (if present)

Mobile phase: Transfer 390 mg of ammonium acetate to a 1000-mL volumetric flask, add 100 mL of water, and dissolve by swirling. Add 6 mL of glacial acetic acid and 300 mL of methanol, dilute with water to volume, and mix. Pass this solution through a filter of 0.5- μ m or finer pore size.

Standard solution: 1 mg/mL of sodium benzoate in water. To 5.0 mL of this solution add 40 mL of methanol, and dilute with water to 100 mL. Pass this solution through a filter of 0.5- μ m or finer pore size.

Sample solution: Transfer about 5 g of well-shaken Rectal Suspension to a 100-mL volumetric flask. Add 40 mL of methanol, dilute with water to volume, and mix. Pass the solution through a filter of 0.5- μ m or finer pore size.

Chromatographic system

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25.0-cm; packing L7

Flow rate: 1.5 mL/min

Injection volume: 15 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage (w/w) of sodium benzoate in the Rectal Suspension taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (10/W)$$

r_U = peak response of sodium benzoate from the *Sample solution*

r_S = peak response of sodium benzoate from the *Standard solution*

C_S = concentration of sodium benzoate in the *Standard solution* (mg/mL)

W = weight of Rectal Suspension taken (g)

Acceptance criteria: 0.05%–0.125%

PERFORMANCE TESTS

• UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Buffer: Transfer 7.1 g of anhydrous dibasic sodium phosphate and 6.9 g of monobasic sodium phosphate to a 1000-mL volumetric flask, add 500 mL of water, and swirl to dissolve. Add 7.5 mL of a solution of tetrabutylammonium hydroxide in methanol (1 in 4), dilute with water to volume, and mix.

Mobile phase: Methanol and Buffer (15:85)

System suitability solution: 0.25 mg/mL of 4-aminosalicylic acid and 0.4 mg/mL of USP Mesalamine RS in *Mobile phase*

Standard stock solution: Transfer about 100 mg of USP Mesalamine RS to a 50-mL volumetric flask, add 15 mL of 2 N hydrochloric acid, and dissolve by swirling. Dilute with 2 N hydrochloric acid to volume, and mix.

Standard solution: Add 5 mL of 2 N sodium hydroxide to 5.0 mL of the *Standard stock solution*, and dilute with *Mobile phase* to 25 mL. Pass this solution through a filter of 0.5- μ m or finer pore size.

Sample stock solution: Transfer, with the aid of 2 N hydrochloric acid, the contents of a container of Rectal Suspension to a 200-mL volumetric flask. Add 2 N hydrochloric acid to obtain about 160 mL of solution, and shake for 10 min. Dilute with 2 N hydrochloric acid to volume, and mix.

Sample solution: Transfer a suitable volume of the *Sample stock solution*, nominally equivalent to 40 mg of mesalamine, to a 100-mL volumetric flask. Add a volume of 2 N hydrochloric acid, equal to the added volume of the *Sample stock solution*, dilute with *Mobile phase* to volume, and mix. Pass this solution through a suitable filter of 0.5- μ m or finer pore size.

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm \times 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 15 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between 4-aminosalicylic acid and mesalamine, *System suitability solution*

Tailing factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) in the container of Rectal Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of mesalamine from the *Sample solution*

r_S = peak response of mesalamine from the *Standard solution*

C_S = concentration of USP Mesalamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mesalamine in the *Sample solution* (mg/mL)

Acceptance criteria: Meets the requirements

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase: Dissolve 1.36 g of monobasic potassium phosphate and 2.2 g of sodium 1-octanesulfonate in 890 mL of water, and adjust with phosphoric acid to a pH of 2.2. Pass through a filter of 0.5- μ m or finer pore size. To the filtrate add 80 mL of methanol and 30 mL of acetonitrile.

Standard solution: 1 μ g/mL each of USP Mesalamine RS and 3-aminosalicylic acid in *Mobile phase*

Sample solution: Transfer a volume of Rectal Suspension, previously well shaken, nominally equivalent to 100 mg of mesalamine, to a beaker. Add water to give a volume of about 80 mL, and adjust with phosphoric acid to a pH of 2.0. Sonicate briefly to dissolve, transfer

to a 100-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Flow rate: 1.2 mL/min

Injection volume: 20 μL

Run time: 3 times the retention time of mesalamine

System suitability

Sample: Standard solution

[NOTE—The relative retention times for mesalamine and 3-aminosalicylic acid are about 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 2 between mesalamine and 3-aminosalicylic acid

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Rectal Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of any individual impurity from the Sample solution

r_s = peak response of mesalamine from the Standard solution

C_s = concentration of USP Mesalamine RS in the Standard solution (μg/mL)

C_u = nominal concentration of mesalamine in the Sample solution (μg/mL)

Acceptance criteria

Individual impurities: NMT 0.2%

Total impurities: NMT 1.0%

SPECIFIC TESTS

• PH (791)

Sample solution: Dilute the Rectal Suspension 1 to 10 with water.

Acceptance criteria: 3.5–5.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP Mesalamine RS

Mesalamine Delayed-Release Tablets

DEFINITION

Mesalamine Delayed-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of mesalamine ($C_7H_7NO_3$).

IDENTIFICATION

• **A. INFRARED ABSORPTION (197K)**

Sample solution: To about 50 mL of water add a quantity of finely powdered Tablets, nominally equivalent to about 800 mg of mesalamine. Boil the mixture for about 5 min, with constant stirring. Filter the hot solution, and allow the filtrate to cool. Collect the precipitated crystals, and dry at about 110°.

Acceptance criteria: Meet the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Dissolve 4.3 g of sodium 1-octanesulfonate in 1 L of water. Adjust with phosphoric acid to a pH

of 2.15, pass through a filter of 0.45-μm or finer pore size, and degas.

System suitability stock solution:

Transfer about 20 mg each of 3-aminosalicylic acid and USP Salicylic Acid RS to a 200-mL volumetric flask. Dissolve in 50 mL of 1 N hydrochloric acid, sonicate to dissolve, dilute with water to volume, and mix.

System suitability solution:

0.01 mg/mL each of 3-aminosalicylic acid and salicylic acid in water from the System suitability stock solution

Standard stock solution:

Transfer about 25 mg of USP Mesalamine RS to a 25-mL volumetric flask. Dissolve in 5 mL of 0.25 N hydrochloric acid, sonicate to dissolve, dilute with water to volume, and mix.

Standard solution: Transfer 10.0 mL of the Standard stock solution and 5.0 mL of the System suitability solution to a 50-mL volumetric flask. Dilute with water to volume, mix, and pass through a filter of 0.5-μm or finer pore size.

Sample solution: Pipet a 25.0-mL aliquot of the Sample solution, obtained as directed in Organic Impurities, into a 100-mL volumetric flask. Dilute with water to volume, and pass through a filter of 0.5-μm or finer pore size.

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV 230 nm

Columns

Precolumns: Two 4.6-mm × 3.0-cm; each containing 10-μm packing L1 and located between the pump and the injector

Analytical: 4.6-mm × 3.3-cm; 3-μm base-deactivated packing L1

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitability

Sample: Standard solution

Suitability requirements

Resolution: NLT 2 between mesalamine and salicylic acid or 3-aminosalicylic acid

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of mesalamine from the Sample solution

r_s = peak response of mesalamine from the Standard solution

C_s = concentration of USP Mesalamine RS in the Standard solution (mg/mL)

C_u = nominal concentration of mesalamine in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION (711)**

Solution A: Transfer about 43.35 g of monobasic potassium phosphate and 1.65 g of sodium hydroxide to a 2-L volumetric flask. Dissolve in and dilute with water to volume, and mix. Adjust with 1 N sodium hydroxide or phosphoric acid to a pH of 6.0, and mix.

Solution B: Transfer 133.6 g of sodium hydroxide to a 2-L volumetric flask, dissolve in and dilute with water to volume, and mix.

Medium

Acid stage: 500 mL of 0.1 N hydrochloric acid

Buffer stages: 900 mL of *Solution A*

Apparatus 2

Acid stage: 100 rpm

Buffer stage 1: 100 rpm

Buffer stage 2: 50 rpm

Times

Acid stage: 2 h

Buffer stage 1: 1 h

Buffer stage 2: 90 min

Acid stage

After 2 h of operation, withdraw an aliquot of the fluid, discard the remaining solution, and retain the Tablets in proper order so that each will be returned later to its respective vessel. Blot the Tablets with a paper towel to dry, and proceed immediately as directed in *Buffer stage 1*.

Standard solution: A known concentration of USP Mesalamine RS in *Medium*, equivalent to about 1% of the labeled amount of mesalamine ($C_7H_7NO_3$)

Sample solution: Filter portions of the solution under test, and suitably dilute with *Medium*, if necessary.

Analysis: Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) dissolved by comparing the UV maximum absorbance at about 302 nm of the *Sample solution* with that of the *Standard solution*.

Tolerances: See *Table 1*. Continue testing through all levels unless the results conform at an earlier level.

Buffer stage 1

[NOTE—Use *Solution A* that has been equilibrated to a temperature of $37 \pm 0.5^\circ$.]

Transfer *Solution A* to each of the dissolution vessels, and place each Tablet from the *Acid stage* into its respective vessel. After 1 h, remove a 50-mL aliquot, and proceed immediately as directed in *Buffer stage 2*.

Standard solution: A known concentration of USP Mesalamine RS in *Medium*, equivalent to about 1% of the labeled amount of mesalamine ($C_7H_7NO_3$)

Sample solution: Filter portions of the solution under test, and suitably dilute with *Medium*, if necessary.

Analysis: Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) dissolved by comparing the UV maximum absorbance at about 330 nm of the *Sample solution* with that of the *Standard solution*.

Tolerances: See *Table 1*. Continue testing through all levels unless the results conform at an earlier level.

Table 1

Level	Number Tested	Acceptance Criteria
L1	6	No individual value exceeds 1% dissolved.
L2	6	Average of the 12 units ($L_1 + L_2$) is NMT 1% dissolved, and no individual unit is greater than 10% dissolved.
L3	12	Average of the 24 units ($L_1 + L_2 + L_3$) is NMT 1% dissolved, and NMT one individual unit is greater than 10% dissolved.

Buffer stage 2

Add 50 mL of *Solution B* to each dissolution vessel to adjust to a pH of 7.2, and continue the run.

Standard solution: A known concentration of USP Mesalamine RS in *Medium*

Sample solution: Filter portions of the solution under test, and suitably dilute with *Medium*, if necessary.

Analysis: Calculate the percentage of the labeled amount of mesalamine ($C_7H_7NO_3$) dissolved by comparing the UV maximum absorbance at about 332 nm of the *Sample solution* with that of the *Standard solution*.

Tolerances: NLT 80% (Q) of the labeled amount of mesalamine ($C_7H_7NO_3$) is dissolved. The requirements are met if the quantities dissolved from the product conform to *Acceptance Table 4* in (711). Continue testing through all levels unless the results conform at an earlier level.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Weight Variation*

IMPURITIES• **ORGANIC IMPURITIES**

Mobile phase, System suitability stock solution, System suitability solution, Standard stock solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Sample solution: Transfer a portion nominally equivalent to about 400 mg of mesalamine, from NLT 20 finely powdered Tablets, to a 500-mL volumetric flask. Add 50 mL of 1 N hydrochloric acid, and sonicate to dissolve. Shake by mechanical means for 10 min, dilute with water to volume, mix, and pass through a filter of 0.5- μ m or finer pore size.

[NOTE—Use an aliquot of this solution for the preparation of the *Sample solution* in the *Assay*.]

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = peak response for each impurity

r_T = sum of all the peak responses

Acceptance criteria

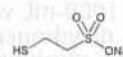
Individual impurity: The largest secondary peak is NMT 1.0% of the total area.

Any other individual impurity: NMT 0.5%

Total impurities: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Mesalamine RS
USP Salicylic Acid RS

Mesna

$C_2H_5NaO_3S_2$ 164.18
Ethanesulfonic acid, 2-mercapto-, monosodium salt;
Sodium 2-mercaptoethanesulfonate [19767-45-4].

DEFINITION

Mesna contains NLT 96.0% and NMT 102.0% of mesna ($C_2H_5NaO_3S_2$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B. IDENTIFICATION TESTS—GENERAL (191), Sodium**
Acceptance criteria: Meet the requirements

ASSAY• **PROCEDURE**

Sample solution: 120 mg of Mesna in 10 mL of water

Analysis: To the *Sample solution* add 10 mL of 1 M sulfuric acid and 10 mL of 0.1 N iodine VS. Titrate with 0.1 N sodium thiosulfate VS, adding 1 mL of starch TS near the endpoint. Perform a blank determination, and make any necessary corrections (see *Titrimetry* (541)).

Each mL of sodium thiosulfate is equivalent to 16.42 mg of mesna ($C_2H_5NaO_3S_2$).

Acceptance criteria: 96.0%–102.0% on the dried basis

IMPURITIES

• LIMIT OF CHLORIDE

Chloride standard solution: 8.24 µg/mL of sodium chloride in water

Sample solution: 200 mg/mL of Mesna in carbon dioxide-free water

Analysis: To 1 mL of the *Sample solution* and 15 mL of water add 1 mL of 2 M nitric acid. Add the resulting solution to 1 mL of silver nitrate solution (17 g in 1000 mL), and allow to stand for 5 min, protected from light. To 10 mL of the *Chloride standard solution* add 5 mL of water and 1 mL of 2 M nitric acid. To this solution add 1 mL of silver nitrate solution (17 g in 1000 mL) and allow to stand for 5 min, protected from light. When viewed against a dark background, the *Sample solution* is not more turbid than the *Chloride standard solution*.

Acceptance criteria: NMT 250 ppm

• LIMIT OF SULFATE

Diluent: 30% (v/v) ethanol in water

Sulfate standard stock solution: 1.81 mg/mL of potassium sulfate in *Diluent*

Sulfate standard solution: 0.0181 mg/mL of potassium sulfate in *Diluent*, prepared immediately before use from *Sulfate standard stock solution*

Sample solution: Add 5.0 mL of the *Sample solution* prepared as directed in the test for *Limit of Chloride* to a 30-mL volumetric flask, and dilute with water to volume.

Analysis: Add 3 mL of a 250-g/L solution of barium chloride to 4.5 mL of *Sulfate standard solution*. Shake and allow to stand for 1 min. To 2.5 mL of this solution add 15 mL of the *Sample solution* and 0.5 mL of acetic acid. Use 15 mL of this mixture for comparison with 15 mL of the *Sulfate standard solution*, prepared in the same manner, but using the *Sulfate standard solution* instead of the *Sample solution*. After 5 min, any opalescence in the *Sample solution* is not more intense than that in the *Sulfate standard solution*.

Acceptance criteria: NMT 300 ppm

Delete the following:

• **HEAVY METALS**, *Method I* (231): 10 ppm • (Official 1-Jan-2018)

• ORGANIC IMPURITIES

Mobile phase: In a 1000-mL volumetric flask dissolve 2.94 g of potassium dihydrogen phosphate, 2.94 g of dipotassium hydrogen phosphate, and 2.6 g of tetrabutylammonium hydrogen sulfate in about 600 mL of water. Adjust with phosphoric acid to a pH of 2.3, add 335 mL of methanol, and dilute with water to volume.

System suitability solution: 0.18 mg/mL and 0.004 mg/mL of USP Mesna RS and USP Mesna Related Compound A RS, respectively, in *Mobile phase*

Standard solution 1: 8 µg/mL and 120 µg/mL of USP Mesna Related Compound A RS and USP Mesna Related Compound B RS, respectively, in *Mobile phase*

Standard solution 2: 12 µg/mL of USP Mesna RS in *Mobile phase*

Sample solution: 4 mg/mL of Mesna in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 10-µm packing L1

Flow rate: 1 mL/min

Run time: Four times the elution time for mesna

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for mesna and mesna related compound A are about 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 3.0 between mesna and mesna related compound A

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

[NOTE—Identify the peaks using the relative retention times provided in *Table 1*.]

Calculate the percentage of mesna related compound A in the portion of Mesna taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of mesna related compound A from the *Sample solution*

r_s = peak response of mesna related compound A from *Standard solution 1*

C_s = concentration of USP Mesna Related Compound A RS in *Standard solution 1* (mg/mL)

C_u = concentration of Mesna in the *Sample solution* (mg/mL)

Calculate the percentage of mesna related compound B in the portion of Mesna taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of mesna related compound B from the *Sample solution*

r_s = peak response of mesna related compound B from *Standard solution 1*

C_s = concentration of USP Mesna Related Compound B RS in *Standard solution 1* (mg/mL)

C_u = concentration of Mesna in the *Sample solution* (mg/mL)

Calculate the percentage of any specified impurities (thiuronium ethanesulfonic acid, guanidinothiuronium ethanesulfonic acid, and mesna triazine analog) and any unspecified impurities in the portion of Mesna taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of any specified or unspecified individual impurity from the *Sample solution*

r_s = peak response of mesna from *Standard solution 2*

C_s = concentration of USP Mesna RS in *Standard solution 2* (mg/mL)

C_u = concentration of Mesna in the *Sample solution* (mg/mL)

F = relative response factors (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Thiouronium ethanesulfonic acid ^a	0.6	100	0.3
Guanidinetiouronium ethanesulfonic acid ^b	0.6	100	0.3
Mesna triazine analog ^c	0.8	100	0.3
Mesna	1.0	—	—
Mesna related compound A	1.4	—	0.2
Mesna related compound B	2.3	—	3.0
Individual unspecified impurities	—	1.0	0.1
Total unspecified impurities	—	—	0.3

^a 2-(Carbamimidoylthio)ethane-1-sulfonic acid.^b 2-[(N-Carbamimidoylcarbamimidoyl)thio]ethane-1-sulfonic acid.^c 2-[(4,6-Diamino-1,3,5-triazin-2-yl)thio]ethane-1-sulfonic acid.**SPECIFIC TESTS****• LOSS ON DRYING**

Sample: 1 g

Analysis: Dry the Sample under vacuum at a pressure not exceeding 1 mm of mercury at 60° over phosphorus pentoxide for 2 h.

Acceptance criteria: NMT 1.0%

• pH (791)

Sample solution: 100 mg/mL of Mesna in carbon dioxide-free water

Acceptance criteria: 4.5–6.0

ADDITIONAL REQUIREMENTS**• PACKAGING AND STORAGE:** Preserve in a tight container, and store at room temperature.**• USP REFERENCE STANDARDS (11)**

USP Mesna RS

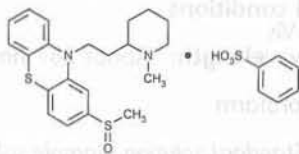
USP Mesna Related Compound A RS

2-(Acetylthio)ethane-1-sulfonic acid.

C₄H₈O₄S₂ 184.22

USP Mesna Related Compound B RS

2,2'-Disulfanediyldis(ethane-1-sulfonic acid).

C₄H₁₀O₆S₄ 282.36**Mesoridazine Besylate**C₂₁H₂₆N₂O₅S₂ · C₆H₅O₃S 544.75

10H-Phenothiazine, 10-[2-(1-methyl-2-piperidinylethyl)-2-(methylsulfonyl)-, (±)-, monobenzenesulfonate.

(±)-10-[2-(1-Methyl-2-piperidinylethyl)-2-(methylsulfonyl)phenothiazine monobenzenesulfonate [32672-69-8].

» Mesoridazine Besylate contains not less than 98.0 percent and not more than 102.0 percent of C₂₁H₂₆N₂O₅S₂ · C₆H₅O₃S, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Mesoridazine Besylate RS

NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

Identification—

A: Infrared Absorption (197M).

B: Ultraviolet Absorption (197U)—

Solution: 10 µg per mL.

Medium: methanol.

Absorptivities at 263 nm, calculated on the dried basis, do not differ by more than 3.0%.

pH (791): between 4.2 and 5.7, in a freshly prepared solution (1 in 100).

Loss on drying (731)—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.2%.

Delete the following:

• **Heavy metals, Method II (231):** 0.002%. (Official 1-Jan-2018)

Selenium (291)—The absorbance of the solution from the Test Solution, prepared with 100 mg of Mesoridazine Besylate and 100 mg of magnesium oxide, is not greater than one-half that from the Standard Solution (0.003%).

Ordinary impurities (466)—

Test solution: a solution in methanol having a known concentration of 14.1 mg per mL equivalent to 10 mg of mesoridazine per mL.

Standard solution: methanol.

Eluant: a mixture of chloroform, isopropyl alcohol, and ammonium hydroxide (87:12:1).

Visualization: 3, followed by spraying with 3% (v/v) aqueous hydrogen peroxide.

Application volume: 10 µL.

Limit: 3.0%.

Assay—Dissolve about 150 mg of Mesoridazine Besylate, accurately weighed, in 70 mL of acetic anhydride, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.24 mg of C₂₁H₂₆N₂O₅S₂ · C₆H₅O₃S.

Mesoridazine Besylate Injection**DEFINITION**

Mesoridazine Besylate Injection is a sterile solution of Mesoridazine Besylate in Water for Injection. It contains mesoridazine besylate (C₂₁H₂₆N₂O₅S₂ · C₆H₅O₃S) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of mesoridazine (C₂₁H₂₆N₂O₅S₂).

Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

IDENTIFICATION**• A. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181)**

Sample solution: Dilute a volume of Injection, equivalent to 50 mg of mesoridazine besylate, with 0.01 N hydrochloric acid to 25 mL.

Analysis: Proceed as directed in the chapter, beginning with "Transfer the liquid to a separator".

Acceptance criteria: Meets the requirements

ASSAY

PROCEDURE

Conduct this procedure with minimum exposure to light.

Standard solution and Sample solution: Proceed with Injection as directed in *Salts of Organic Nitrogenous Bases* (501), except use 1.0 mL each of the *Standard Preparation* and the *Assay Preparation* in the *Procedure*.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: Maximum at about 262 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mesoridazine ($C_{21}H_{26}N_2O_5$) in the portion of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Mesoridazine Besylate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of mesoridazine besylate in the *Sample solution* ($\mu\text{g/mL}$)

M_{r1} = molecular weight of mesoridazine, 386.59

M_{r2} = molecular weight of mesoridazine besylate, 544.75

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 7.0 USP Endotoxin Units/mg of mesoridazine besylate.

• **pH** (791): 4.0–5.0

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, protected from light.

• **USP REFERENCE STANDARDS** (11)

USP Endotoxin RS

USP Mesoridazine Besylate RS

Mesoridazine Besylate Oral Solution

DEFINITION

Mesoridazine Besylate Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of mesoridazine ($C_{21}H_{26}N_2O_5$).

Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

IDENTIFICATION

A.

Conduct this test without exposure to daylight and with the minimum necessary exposure to artificial light.

Standard solution: 14 mg/mL of USP Mesoridazine Besylate RS in methanol

Sample solution: Transfer 4.0 mL of Oral Solution into a separator, add 6 mL of 1 N sodium hydroxide and 10 mL of chloroform, shake for 2 min, and filter the chloroform layer through anhydrous sodium sulfate into a small, glass-stoppered conical flask.

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μL

Developing solvent system: To a separator add benzene, alcohol, and ammonium hydroxide (10:2:1). Shake, and allow the layers to separate. Use the upper layer.

Spray reagent: Perchloric acid and water (15:85)

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the spots to dry and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in a fume hood. Spray the plate with *Spray reagent*, and heat at 80° for 2 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in R_f value and color to that of the *Standard solution*.

ASSAY

PROCEDURE

Conduct this procedure with the minimum necessary exposure to light.

Standard stock solution: 0.14 mg/mL of USP Mesoridazine Besylate RS in chloroform prepared as follows. Transfer 14 mg of USP Mesoridazine Besylate RS to a 125-mL separator containing 30 mL of water. Render the solution alkaline with 10 mL of 1 N sodium hydroxide, and extract with three 30-mL portions of chloroform. Filter the extracts through anhydrous sodium sulfate into a 100-mL volumetric flask. Rinse the filter with small portions of chloroform, collecting the rinsings in the volumetric flask, and dilute with chloroform to volume.

Standard solution: 0.014 mg/mL of USP Mesoridazine Besylate RS from a suitable volume of *Standard stock solution* and chloroform

Sample stock solution: Nominally 1 mg/mL of mesoridazine in chloroform prepared as follows. Pipet a volume of Oral Solution, equivalent to 100 mg of mesoridazine, into a separator containing 30 mL of water. Render the solution alkaline with 10 mL of 1 N sodium hydroxide, and extract with three 30-mL portions of chloroform. Filter the extracts through anhydrous sodium sulfate into a 100-mL volumetric flask. Rinse the filter with small portions of chloroform, collecting the rinsings in the volumetric flask, and dilute with chloroform to volume.

Sample solution: Nominally 0.1 mg/mL of mesoridazine from a suitable volume of *Sample stock solution* and chloroform

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: About 267 nm

Cell: 1 cm

Blank: Chloroform

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of labeled amount of mesoridazine ($C_{21}H_{26}N_2O_5$) in the portion of Oral Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Mesoridazine Besylate RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of mesoridazine in the *Sample solution* ($\mu\text{g/mL}$)

M_{r1} = molecular weight of mesoridazine, 386.59

M_{r2} = molecular weight of mesoridazine besylate, 544.75

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DELIVERABLE VOLUME** (698)
For multiple-unit containers
Acceptance criteria: Meets the requirements
- **UNIFORMITY OF DOSAGE UNITS** (905)
For single-unit containers
Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **ALCOHOL DETERMINATION**, Method I (611): 0.25%–1.0% of the labeled amount of alcohol (C_2H_5OH) is found.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at a temperature not exceeding 25°.
- **LABELING:** Label it to indicate that it is to be diluted to the appropriate strength with water or other suitable fluid before administration.
- **USP REFERENCE STANDARDS** (11)
USP Mesoridazine Besylate RS

Mesoridazine Besylate Tablets

DEFINITION

Mesoridazine Besylate Tablets contain mesoridazine besylate ($C_{21}H_{26}N_2O_5 \cdot C_6H_5O_3S$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of mesoridazine ($C_{21}H_{26}N_2O_5$).

Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** (181):
Meet the requirements

ASSAY

PROCEDURE

Mobile phase: Acetonitrile, triethylamine, and water (850:1:150)

Standard solution: 0.35 mg/mL of USP Mesoridazine Besylate RS in methanol

System suitability solution: 0.025 mg/mL of thioridazine hydrochloride in *Standard solution*

Sample solution: Nominally 0.25 mg/mL of mesoridazine prepared as follows. Transfer an amount of powder equivalent to NLT 50 mg of mesoridazine, from NLT 20 powdered Tablets, to a suitable volumetric flask. Add 75% of the flask volume of methanol, shake by mechanical means for 15 min, and dilute with methanol to volume. Sonicate for 30 min, and allow dispersed material to settle. Filter through a 0.25- μ m disk, discarding the first 20 mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm \times 25-cm; packing L1

Flow rate: 2.5 mL/min

Injection volume: 10 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 1.0 between mesoridazine besylate and thioridazine hydrochloride, *System suitability solution*

Column efficiency: NLT 750 theoretical plates for the mesoridazine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mesoridazine ($C_{21}H_{26}N_2O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mesoridazine Besylate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mesoridazine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of mesoridazine, 386.59

M_{r2} = molecular weight of mesoridazine besylate, 544.75

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: 0.01 N hydrochloric acid; 1000 mL

Apparatus 2: 100 rpm

Time: 60 min

Standard solution: USP Mesoridazine Besylate RS in *Medium*. [NOTE—A volume of methanol not exceeding 1% of the final total volume may be used to prepare the *Standard solution*.]

Sample solution: Filter the solution under test, suitably diluted with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 261 nm

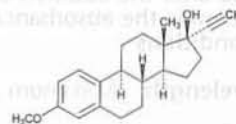
Tolerances: NLT 80% (Q) of the labeled amount of mesoridazine ($C_{21}H_{26}N_2O_5$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Preserve Tablets with an opaque coating in well-closed containers.
- **USP REFERENCE STANDARDS** (11)
USP Mesoridazine Besylate RS

Mestranol



$C_{21}H_{26}O_2$ 310.43
19-Norpregna-1,3,5(10)-trien-20-yn-17-ol, 3-methoxy-, (17 α)-;
3-Methoxy-19-nor-17 α -pregna-1,3,5(10)-trien-20-yn-17-ol
[72-33-3].

DEFINITION

Mestranol contains NLT 97.0% and NMT 102.0% of mestranol ($C_{21}H_{26}O_2$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B. ULTRAVIOLET ABSORPTION (197U)**
Sample solution: 100 µg/mL of Mestranol in methanol
Acceptance criteria: Meets the requirements
- **C.**
Standard solution: 1 mg/mL of USP Mestranol RS in chloroform
Sample solution: 1 mg/mL of Mestranol in chloroform
Chromatographic system
(See *Chromatography* (621), *Thin-Layer Chromatography*.)
Mode: TLC
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing solvent system: Chloroform and dehydrated alcohol (29:1)
Spray reagent: Prepare as directed in *Solution A* in the Assay.

Analysis

Samples: *Standard solution* and *Sample solution*
Apply both solutions to a line parallel to and 2.5 cm from the bottom edge of a thin-layer chromatographic plate. Place the plate in the *Developing solvent system*. Develop the chromatogram until the solvent front has moved about 15 cm from the origin. Remove the plate from the chamber, allow the solvent to evaporate, and spray with *Spray reagent*. Heat the plate in an oven at 105° for 5 min, and observe under long-wavelength UV light.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY• **PROCEDURE**

Solution A: Pipet 30 mL of methanol into a 100-mL volumetric flask contained in an ice bath. Add slowly and cautiously and with continuous stirring about 65 mL of sulfuric acid, taking care that the temperature remains below 15°. Allow the solution to warm to room temperature, and dilute with sulfuric acid to volume.

Standard solution: 5 µg/mL of USP Mestranol RS in chloroform

Sample solution: 5 µg/mL of Mestranol in chloroform

Analysis: Pipet 4 mL each of the *Standard solution* and the *Sample solution* into separate glass-stoppered, 25-mL conical flasks. Evaporate the solutions under a slow current of air, without the aid of heat, to dryness. Dissolve the residue in 0.3 mL of methanol. Place the flasks in a water bath maintained at a temperature of 25°, and pipet into each, with constant swirling, 10 mL of *Solution A*. Insert the stoppers in the flasks. At 6 min, accurately timed after the addition of *Solution A*, concomitantly determine the absorbances of the solutions.

Instrumental conditions

Mode: Vis

Analytical wavelength: Maximum at about 545 nm

Cell: 1 cm

Blank: *Solution A*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of mestranol ($C_{21}H_{26}O_2$) in the portion of Mestranol taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

- A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of USP Mestranol RS in the *Standard solution* (µg/mL)
 C_U = concentration of Mestranol in the *Sample solution* (µg/mL)

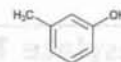
Acceptance criteria: 97.0%–102.0% on the dried basis

SPECIFIC TESTS

- **LOSS ON DRYING (731)**
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 1.0%
- **MELTING RANGE OR TEMPERATURE (741):** 146°–154°, but the range between beginning and end of melting does not exceed 4°.
- **OPTICAL ROTATION, Specific Rotation (781S)**
Sample solution: 20 mg, previously dried, per mL, in dioxane
Acceptance criteria: +2° to +8°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**
USP Mestranol RS

Metacresol

C_7H_8O

3-Methylphenol;

3-Hydroxytoluene [108-39-4].

108.14

DEFINITION

Metacresol contains NLT 98.0% and NMT 102.0% of metacresol (C_7H_8O).

IDENTIFICATION

- **A. INFRARED ABSORPTION (197F)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY• **PROCEDURE**

Internal standard solution: 1 mg/mL of USP Phenol RS in methanol

System suitability solution: 5 mg/mL each of metacresol, orthocresol, and paracresol in methanol

Standard solution: 1 mg/mL of USP Metacresol RS in the *Internal standard solution*

Sample solution: 1 mg/mL of Metacresol in the *Internal standard solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame-ionization

Column: 0.25-mm × 30-m; 0.25-µm coating of G7

Temperatures

Injection port: 200°

Detector: 200°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
90	0	90	10
90	2	120	0
120	10	150	3

Carrier gas: Helium

Flow rate: 1.8 mL/min

Injection type: Split ratio of 1:40

Injection volume: 1 μ L

System suitability

Samples: System suitability solution and Standard solution

[NOTE—The relative retention times for phenol, orthocresol, paracresol, and metacresol are about 0.77, 0.85, 0.99, and 1.00, respectively.]

Suitability requirements

Resolution: NLT 1.4 between the paracresol and metacresol peaks, System suitability solution

Relative standard deviation: NMT 1.0% for the peak ratio of metacresol to phenol, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of metacresol (C_7H_8O) in the portion of Metacresol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the metacresol peak response to the phenol peak response from the Sample solution

R_S = ratio of the metacresol peak response to the phenol peak response from the Standard solution

C_S = concentration of USP Metacresol RS in the Standard solution (mg/mL)

C_U = concentration of Metacresol in the Sample solution (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• ORGANIC IMPURITIES

System suitability solution: 5 mg/mL each of metacresol, orthocresol, and paracresol in methanol

Sensitivity solution: 5 μ g/mL of USP Metacresol RS in methanol

Sample solution: 10 mg/mL of Metacresol in methanol

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: GC

Detector: Flame-ionization

Column: 0.25-mm \times 30-m; 0.25- μ m coating of G7

Temperatures

Injection port: 200°

Detector: 200°

Column: See Table 2.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
90	0	90	10
90	2	150	15

Carrier gas: Helium

Flow rate: 1.8 mL/min

Injection type: Split ratio of 1:40

Injection volume: 1 μ L

System suitability

Samples: System suitability solution and Sensitivity solution

Suitability requirements

Resolution: NLT 1.4 between the paracresol and metacresol peaks, System suitability solution

Signal-to-noise ratio: NLT 10, Sensitivity solution

Analysis

Sample: Sample solution

Calculate the percentage of each individual impurity in the portion of Metacresol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of each individual impurity from the Sample solution

r_T = sum of all the peak areas from the Sample solution

Acceptance criteria: See Table 3. Disregard any peak less than 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Orthocresol	0.85	0.5
Paracresol	0.99	0.5
Metacresol	1.00	—
Any unspecified impurity	—	0.1
Total impurities	—	1.0

SPECIFIC TESTS

• CLARITY OF SOLUTION

A.

Analysis: Add 10 mL of Metacresol to 10 mL of hexane, and mix.

Acceptance criteria: A clear solution is obtained.

B.

Analysis: Add 1.0 mL of Metacresol to 20 mL of 1 N sodium hydroxide, and mix.

Acceptance criteria: A clear solution is obtained.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• USP REFERENCE STANDARDS (11)

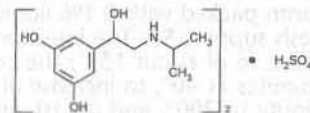
USP Metacresol RS

USP Phenol RS

Phenol.

C_6H_6O 94.11

Metaproterenol Sulfate



$(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ 520.59

1,3-Benzenediol, 5-[1-hydroxy-2-(1-methylethyl)amino]-ethyl-, (\pm)-, sulfate (2:1) (salt).

(\pm)-3,5-Dihydroxy- α -[(isopropylamino)methyl]benzyl alcohol sulfate (2:1) [5874-97-5].

» Metaproterenol Sulfate contains not less than 98.0 percent and not more than 102.0 percent of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$, calculated on the anhydrous, isopropyl alcohol-free, and methanol-free basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Metaproterenol Sulfate RS

Identification—**A:** *Infrared Absorption* (197K).**B:** To a solution of 10 mg in 1 mL of water add 1 drop of ferric chloride TS: a violet color is produced.**C:** It responds to the tests for *Sulfate* (191).**D:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for metaproterenol, the retention time of which corresponds with that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.**pH** (791): between 4.0 and 5.5, in a solution containing 100 mg per mL.**Water Determination, Method I** (921): not more than 2.0%.**Residue on ignition** (281): not more than 0.1%.**Delete the following:****•Heavy metals, Method II** (231): 0.001%. (Official 1-Jan-2018)**Iron** (241)—Dissolve 2.0 g in 45 mL of water, add 2 mL of hydrochloric acid, and mix: the limit is 5 ppm.**Limit of metaproterenone sulfate**—Its absorptivity (see *Ultraviolet-Visible Spectroscopy* (857)) at 328 nm, determined in an aqueous solution containing 9.0 mg per mL, is not more than 0.009 (0.1%).**Isopropyl alcohol and methanol—****Isopropyl alcohol standard solution**—Transfer about 0.3 g of isopropyl alcohol, accurately weighed, to a 100-mL volumetric flask containing about 10 mL of water, dilute with water to volume, and mix. Pipet 10 mL of the resulting solution into a 100-mL volumetric flask, add about 85 mL of pyridine, mix, and allow to stand for 1 hour. Dilute with pyridine to volume, and mix. Pipet 5 mL of this solution to a 50-mL volumetric flask, dilute with pyridine to volume, and mix. The solution so obtained contains about 30 µg of isopropyl alcohol per mL.**Methanol standard solution**—Prepare as directed for *Isopropyl alcohol standard solution*, using about 0.1 g of methanol, accurately weighed. The resulting solution contains about 10 µg of methanol per mL.**Test preparation**—Transfer about 1 g of Metaproterenol Sulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in about 2 mL of water, dilute with pyridine to volume, and mix.**Chromatographic system**—The gas chromatograph is equipped with a flame-ionization detector and contains a 2-m × 2-mm column packed with 0.1% liquid phase G25 on 80- to 100-mesh support S7. The injection port is maintained at a temperature of about 150°; the column is programmed for 2 minutes at 40°, to increase at a rate of about 15° per minute to 200°, and for 10 minutes at 200°; the detector is maintained at about 250°; and helium is used as the carrier gas at a flow rate of about 15 mL per minute.**Procedure**—Inject equal volumes (about 2 µL) of the *Test preparation*, the *Isopropyl alcohol standard solution*, and the *Methanol standard solution* successively into the gas chromatograph. Measure the responses of the isopropyl alcohol peak and the methanol peak in each chromatogram. Determine the quantities, in mg, of isopropyl alcohol and methanol in the portion of Metaproterenol Sulfate taken by the formula:

$$0.1C(r_u / r_s)$$

in which *C* is the concentration, in µg per mL, of isopropyl alcohol or methanol in the *Isopropyl alcohol standard solution* or the *Methanol standard solution*; and *r_u* and *r_s* are the re-sponses of the respective analytes in the *Test preparation* and of the corresponding *Isopropyl alcohol standard solution* or *Methanol standard solution*: not more than 0.3% of isopropyl alcohol and not more than 0.1% of methanol are found.**Assay—****Mobile phase**—Dissolve 11.9 g of anhydrous dibasic sodium phosphate in water to make 1000 mL of solution, and mix (*Solution A*). Dissolve 9.1 g of monobasic potassium phosphate in water to make 1000 mL of solution, and mix (*Solution B*). Mix 735 mL of *Solution A* and 140 mL of *Solution B*, add 125 mL of methanol, and mix. Filter and degas this solution before use.**Standard preparation**—Dissolve an accurately weighed quantity of USP Metaproterenol Sulfate RS in 0.01 N hydrochloric acid to obtain a solution having a known concentration of about 2 mg per mL.**Assay preparation**—Transfer about 100 mg of Metaproterenol Sulfate, accurately weighed, to a 50-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm × 5-cm guard column that contains packing L7 and a 4.6-mm × 25-cm analytical column that contains 10-µm packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 500 theoretical plates, the tailing factor for the analyte peak is not more than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of (C₁₁H₁₇NO₃)₂ · H₂SO₄ in the portion of Metaproterenol Sulfate taken by the formula:

$$50C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Metaproterenol Sulfate RS in the *Standard preparation*, and *r_u* and *r_s* are the peak responses from the *Assay preparation* and the *Standard preparation*, respectively.**Metaproterenol Sulfate Inhalation Aerosol**» Metaproterenol Sulfate Inhalation Aerosol is a suspension of microfine Metaproterenol Sulfate in fluorochlorohydrocarbon propellants in a pressurized container. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of metaproterenol sulfate [(C₁₁H₁₇NO₃)₂ · H₂SO₄].**Packaging and storage**—Preserve in small, nonreactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.**USP Reference standards (11)—**

USP Metaproterenol Sulfate RS

Identification—The UV absorption spectrum of the solution from the *Assay preparation*, obtained as directed in the *Assay*, exhibits maxima and minima at the same wavelengths as that of the *Standard preparation* prepared as directed in the *Assay*.

Delivered dose uniformity over the entire contents: meets the requirements for *Metered-Dose Inhalers* under *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* (601).

PROCEDURE FOR DOSE UNIFORMITY—

Standard preparation—Using a suitable quantity of USP Metaproterenol Sulfate RS, accurately weighed, prepare a solution in 0.01 N hydrochloric acid to obtain a solution having a known concentration of 0.05 mg per mL.

Test preparation—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of 0.01 N hydrochloric acid, and quantitatively transfer the rinsings to a 25-mL volumetric flask. Dilute with 0.01 N hydrochloric acid to volume, and mix.

Procedure—Transfer 20.0 mL portions of the *Standard preparation*, the *Test preparation*, and 0.01 N hydrochloric acid to serve as a blank to separate centrifuge tubes. Add 10.0 mL of chloroform to each, shake by mechanical means for 5 minutes, and separate the layers by centrifuging for 5 minutes. Determine the absorbances of the respective aqueous layers in 1-cm cells, at the wavelength of maximum absorbance at about 276 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of metaproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$ contained in the minimum dose taken by the formula:

$$12.5CN(A_U / A_S)$$

in which *C* is the concentration, in mg per mL, of USP Metaproterenol Sulfate RS in the *Standard preparation*; *N* is the number of sprays discharged to obtain the minimum dose; and *A_U* and *A_S* are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

Particle size—Prime the valve of an Inhalation Aerosol container by alternately shaking and firing it several times, and then actuate one measured spray onto a clean, dry microscope slide held 5 cm from the end of the oral inhalation actuator, perpendicular to the direction of the spray. Carefully rinse the slide with about 2 mL of chloroform, and allow to dry. Examine the slide under a microscope equipped with a calibrated ocular micrometer, using 450× magnification. Focus on the particles of 25 fields of view near the center of the test specimen pattern, and note the size of the great majority of individual particles: they are less than 5 μm along the longest axis. Record the number and size of all individual crystalline particles (not agglomerates) more than 10 μm in length measured along the longest axis: not more than 10 such particles are observed.

Water—Transfer the contents of a weighed container to the titration vessel by attaching the valve stem to an inlet tube. Weigh the empty container and determine the weight of the specimen taken. The water content, determined by *Method I* under *Water Determination* (921), is not more than 0.075%.

Assay—Cool an accurately weighed Inhalation Aerosol container for 10 minutes in a bath consisting of a mixture of acetone and solid carbon dioxide. Cut the valve from the aerosol container and allow the container to warm to room temperature. When most of the propellants have evaporated, transfer the residue in the container to a 250-mL separator with the aid of 30 mL of chloroform and 50 mL of 0.01 N hydrochloric acid. Reserve the valve and the empty container. Shake the separator for 1 minute and allow the phases to separate. Transfer the chloroform phase to a second 250-mL separator and the aqueous phase to a 250-mL volumetric flask. Wash the chloroform phase with two 50-mL portions of 0.01 N hydrochloric acid, add the washings to the 250-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Transfer an accurately measured volume of this stock solution, equivalent to about 10 mg of metaproterenol sulfate, to a 100-mL volumetric

flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Dissolve an accurately weighed quantity of USP Metaproterenol Sulfate RS in 0.01 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 100 μg per mL. Concomitantly determine the absorbances of both solutions at the wavelength of maximum absorbance at about 276 nm, with a suitable spectrophotometer, using 0.01 N hydrochloric acid as the blank. Rinse the empty aerosol container and the valve with water and dry them at 105° for 10 minutes, allow to cool, and weigh. Subtract the weight thus obtained from the original weight of the Inhalation Aerosol container to obtain the weight of the Inhalation Aerosol taken. Calculate the quantity, in mg, of metaproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$ in each mL of the Inhalation Aerosol taken by the formula:

$$25(C/V)(d/W)(A_U/A_S)$$

in which *C* is the concentration, in μg per mL, of USP Metaproterenol Sulfate RS in the Standard solution, *V* is the volume, in mL, of stock solution taken, *W* is the weight, in g, of the Inhalation Aerosol taken, and *A_U* and *A_S* are the absorbances of the solution from the Inhalation Aerosol and the Standard solution, respectively. [The density, *d*, is determined as follows: Weigh a known volume (*v*) of the Inhalation Aerosol in a suitable 5-mL gas-tight syringe equipped with a linear valve. Calibrate the volume of the syringe by filling to the 5-mL mark with dichlorotetrafluoroethane withdrawn from a plastic-coated glass vial sealed with a neoprene multiple-dose rubber stopper and an aluminum seal, using 1.456 g per mL as the density of the calibrating liquid. Maintain the dichlorotetrafluoroethane, the Inhalation Aerosol sample, and the syringe (protected from becoming wet) at 25° in a water bath. Obtain the sample, equivalent to the same volume as that obtained during the sampling procedure, from the Inhalation Aerosol by means of a sampling device consisting of a replaceable rubber septum engaged in the plate threads at one end of a threaded fitting, the opposite end of which contains a sharpened tube capable of puncturing the aerosol container, and a rubber gasket around the tube to prevent leakage of the container contents after puncture.* Calculate the density taken by the formula:

$$w/v$$

in which *w* is the weight of the volume, *v*, of the Inhalation Aerosol taken.]

Metaproterenol Sulfate Inhalation Solution

» Metaproterenol Sulfate Inhalation Solution is a sterile solution of Metaproterenol Sulfate in Purified Water. It may contain Sodium Chloride. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of metaproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$.

Packaging and storage—Store in small, tight containers that are well-filled or otherwise protected from oxidation. Protect from light.

Labeling—Label it to indicate that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

* A suitable sampling system is available from Alltek Associates, P. O. Box 498, Arlington Heights, IL 60006.

USP Reference standards (11)—

USP Metaproterenol Sulfate RS

Color and clarity—

Standard solution—Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Visually examine a portion of the Inhalation Solution (*Test solution*) in a suitable clear glass test tube against a white background: it is not pinkish, and it contains no precipitate. If any yellow color is observed in the *Test solution*, concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm: the absorbance of the *Test solution* does not exceed that of the *Standard solution*.

Identification—

A: Apply 4 μ L of the Inhalation Solution and 4 μ L of an aqueous solution of USP Metaproterenol Sulfate RS containing about 50 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of the upper layer of a freshly prepared mixture of butyl alcohol, water, and formic acid (50:25:7) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the R_f value of the principal spot obtained from the Inhalation Solution corresponds to that obtained from the *Standard solution*.

B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for metaproterenol, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Sterility Tests (71): meets the requirements.

pH (791): between 2.8 and 4.0.

Assay—

Mobile phase, Standard preparation, and Chromatographic system—Prepare as directed in the *Assay* under *Metaproterenol Sulfate*.

Assay preparation—Transfer an accurately measured volume of Inhalation Solution, equivalent to about 200 mg of metaproterenol sulfate, to a 100-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Metaproterenol Sulfate*. Calculate the quantity, in mg, of metaproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$ in each mL of the Inhalation Solution taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which V is the volume, in mL, of Inhalation Solution taken; and C , r_U , and r_S are as defined therein.

Metaproterenol Sulfate Oral Solution

» Metaproterenol Sulfate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of metaproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Metaproterenol Sulfate RS

Identification—

A: Transfer a portion of Oral Solution, equivalent to about 10 mg of metaproterenol sulfate, to a separator, and extract with four 30-mL portions of ether, discarding the ether extracts. Apply 10 μ L of the extracted portion of Oral Solution to the lower right corner of a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow to dry. Develop the chromatogram in a solvent system consisting of the lower layer of a well-shaken mixture of dioxane, methylene chloride, alcohol, and ammonium hydroxide (4:4:1:1). Allow the solvent front to move about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry in vacuum at 35° to 40° for 30 minutes. Rotate the plate 90°. At a point about four-fifths of the distance between the initial application of the Oral Solution extract and the solvent front, apply 10 μ L of a *Standard solution* of USP Metaproterenol Sulfate RS in water containing about 2 mg per mL. Proceed as directed in *Identification* test A under *Metaproterenol Sulfate Inhalation Solution*, beginning with "Allow the spots to dry": the R_f value of the principal spot obtained from the Oral Solution corresponds to that obtained from the *Standard solution*.

B: The retention time of the major peak for metaproterenol in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

pH (791): between 2.5 and 4.0, in a solution obtained by mixing 1 volume of Oral Solution and 4 volumes of water.

Assay—

Mobile phase—Mix 10 mL of formic acid and water to make 1000 mL of solution. Filter and degas this solution before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Metaproterenol Sulfate RS in water to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 20 mg of metaproterenol sulfate, to a 100-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 278-nm detector, a 4.6-mm \times 5-cm guard column that contains packing L2, and a 3.9-mm \times 30-cm analytical column that contains packing L1. [NOTE—After use, rinse the analytical column with water and store with water in it.] The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the analyte peak is not more than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 100 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of metaproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$ in each mL of the Oral Solution taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Metaproterenol Sulfate RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken; and r_U and r_S are the peak responses from the *Assay preparation* and the *Standard preparation*, respectively.

Metaproterenol Sulfate Tablets

» Metaproterenol Sulfate Tablets contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of metaproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards (11)—

USP Metaproterenol Sulfate RS

Identification—

A: Powder a number of Tablets, equivalent to about 100 mg of metaproterenol sulfate, add 10 mL of water, stir for about 3 minutes, and centrifuge. Use the clear solution so obtained as the *Test solution*. Dissolve a suitable quantity of USP Metaproterenol Sulfate RS in water to obtain a Standard solution having a concentration of 10 mg per mL. Apply separate 10- μ L portions of the *Test solution* and the Standard solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Proceed as directed in Identification test A under Metaproterenol Sulfate Inhalation Solution, beginning with "Allow the spots to dry": the R_f value of the principal spot obtained from the *Test solution* corresponds to that obtained from the Standard solution.

B: Mix a quantity of powdered Tablets, equivalent to about 20 mg of metaproterenol sulfate, with 5 mL of water, and filter: the filtrate responds to the tests for Sulfate (191).

C: The chromatogram of the Assay preparation obtained as directed in the Assay exhibits a major peak for metaproterenol, the retention time of which corresponds to that exhibited in the chromatogram of the Standard preparation obtained as directed in the Assay.

Dissolution (711)—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm of filtered portions of the solution under test, suitably diluted with Dissolution Medium, if necessary, in comparison with a Standard solution having a known concentration of USP Metaproterenol Sulfate RS in the same Medium.

Tolerances—Not less than 70% (Q) of the labeled amount of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase, Standard preparation, and Chromatographic system—Prepare as directed in the Assay under Metaproterenol Sulfate.

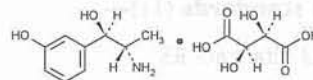
Assay preparation—Transfer 20 Tablets to a 500-mL conical flask. Add an accurately measured volume of 0.01 N hydrochloric acid sufficient to yield a solution containing about 2 mg of metaproterenol sulfate per mL, shake by mechanical means for 30 minutes, and filter. Use the filtrate so obtained as the Assay preparation.

Procedure—Proceed as directed for Procedure in the Assay under Metaproterenol Sulfate. Calculate the quantity, in mg, of metaproterenol sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$ in each Tablet taken by the formula:

$$(CV/20)(r_u/r_s)$$

in which V is the volume, in mL, of 0.01 N hydrochloric acid added; and C , r_u , and r_s are as defined therein.

Metaraminol Bitartrate



$C_9H_{13}NO_2 \cdot C_4H_6O_6$ 317.29

Benzenemethanol, α -(1-aminoethyl)-3-hydroxy-, [R -(R^* , S^*)]-, [R -(R^* , R^*)]-2,3-dihydroxybutanedioate (1:1) (salt).

(-)- α -(1-Aminoethyl)- m -hydroxybenzyl alcohol tartrate (1:1) (salt) [33402-03-8].

» Metaraminol Bitartrate contains not less than 99.0 percent and not more than 100.5 percent of $C_9H_{13}NO_2 \cdot C_4H_6O_6$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards (11)—

USP Metaraminol Bitartrate RS

Identification—

A: Infrared Absorption (197K).

B: To 0.5 mL of a solution (1 in 2000) add 1 mL of Folin-Ciocalteu phenol TS, then add 5 mL of sodium carbonate solution (1 in 10), mix, and allow to stand for 5 minutes: an intense blue color appears (*presence of a phenol*).

C: To 4 mL of a solution (1 in 2000) add 5 mL of pH 9.6 alkaline borate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*), then add about 5 mg of β -naphthoquinone-4-sodium sulfonate, mix until dissolved, and allow to stand for 5 minutes. Add 0.2 mL of benzalkonium chloride solution (1 in 100), mix, add 5 mL of toluene, and shake: the toluene layer turns purple immediately (*distinction from phenylephrine*).

Melting range (741): between 171° and 175°.

Specific rotation (781S): between -31.5° and -33.5° (λ =405 nm).

Test solution: 100 mg per mL, in 0.5 N hydrochloric acid.

pH (791): between 3.2 and 3.5, in a solution (1 in 20).

Loss on drying (731)—Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals, Method I** (231): 0.002%. • (Official 1-Jan-2018)

Assay—Dissolve about 600 mg of Metaraminol Bitartrate, accurately weighed, in 20 mL of glacial acetic acid, warming slightly to effect solution. Cool the solution to room temperature, add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to an emerald-green color. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.73 mg of $C_9H_{13}NO_2 \cdot C_4H_6O_6$.

Metaraminol Bitartrate Injection

» Metaraminol Bitartrate Injection is a sterile solution of Metaraminol Bitartrate in Water for Injection. It contains, in each mL, an amount of metaraminol bitartrate equivalent to not less than 9.0 mg and not more than 11.0 mg of metaraminol ($C_9H_{13}NO_2$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards (11)—

USP Endotoxin RS
USP Metaraminol Bitartrate RS

Identification—

A: Evaporate a 1-mL portion to dryness: the residue so obtained meets the requirements for *Identification test A* under *Metaraminol Bitartrate*.

B: It meets the requirements for *Identification tests B* and *C* under *Metaraminol Bitartrate*.

Bacterial Endotoxins Test (85)—It contains not more than 3.5 USP Endotoxin Units per mg of metaraminol.

pH (791): between 3.2 and 4.5.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

0.0032 M Hexanesulfonate buffer—Mix 600 mg of sodium 1-hexanesulfonate with water to obtain 1000 mL of solution, adjust with phosphoric acid to a pH of 3.0 ± 0.05 , and filter.

Mobile phase—Prepare a suitable degassed and filtered mixture of methanol and **0.0032 M Hexanesulfonate buffer** (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Metaraminol Bitartrate RS in water to obtain a solution having a known concentration of about 0.2 mg of metaraminol per mL.

Assay preparation—Transfer an accurately measured volume of injection, equivalent to about 20 mg of metaraminol, to a 100-mL volumetric flask, dilute with water to volume, and mix.

System suitability preparation—Prepare a solution of propylparaben in alcohol containing 0.4 mg per mL. Mix 1 volume of this solution with 99 volumes of the *Standard preparation*.

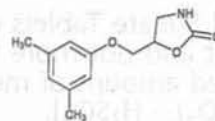
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 264-nm detector and a 4-mm \times 25-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *System suitability preparation* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2600 theoretical plates, the resolution, R , between the metaraminol bitartrate and propylparaben peaks is not less than 3.0 with propylparaben eluting first, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of metaraminol ($C_9H_{13}NO_2$) in each mL of the injection taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of metaraminol represented by the USP Metaraminol Bitartrate RS in the *Standard preparation*; V is the volume, in mL, of injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Metaxalone



$C_{12}H_{15}NO_3$ 221.25
2-Oxazolidinone, 5-[(3,5-dimethylphenoxy)methyl]-;
5-[(3,5-Xylyloxy)methyl]-2-oxazolidinone;
5-[(3,5-Dimethylphenoxy)methyl]-1,3-oxazolidin-2-one
[1665-48-1].

DEFINITION

Metaxalone contains NLT 98.0% and NMT 102.0% of metaxalone ($C_{12}H_{15}NO_3$), calculated on dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197A) or (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Sample solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: 0.68 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.5.

Mobile phase: Methanol and *Buffer* (50:50)

Standard stock solution: 0.5 mg/mL of USP Metaxalone RS prepared as follows. Transfer a suitable quantity of USP Metaxalone RS to a suitable volumetric flask. Add 50% of the flask volume of methanol. Sonicate for 5 min to dissolve. Add 40% of the flask volume of *Buffer*, and mix. Cool to room temperature. Dilute with *Buffer* to volume.

Standard solution: 0.05 mg/mL of USP Metaxalone RS from the *Standard stock solution* in *Mobile phase*

Sample stock solution: 0.5 mg/mL of Metaxalone prepared as follows. Transfer a suitable quantity of Metaxalone to a suitable volumetric flask. Add 50% of the flask volume of methanol. Sonicate for 5 min to dissolve. Add 40% of the flask volume of *Buffer*, and mix. Cool to room temperature. Dilute with *Buffer* to volume.

Sample solution: 0.05 mg/mL of Metaxalone from the *Sample stock solution* in *Mobile phase*

Chromatographic system
(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 226 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: NLT 2 times the retention time of metaxalone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of metaxalone ($C_{12}H_{15}NO_3$) in the portion of Metaxalone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Metaxalone RS in the *Standard solution* (mg/mL)

C_U = concentration of Metaxalone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• RESIDUE ON IGNITION (281): NMT 0.30%

• ORGANIC IMPURITIES, PROCEDURE 1

If metaxalone related compound B, metaxalone related compound C, or *N*-benzyl metaxalone is a known process impurity, *Organic Impurities, Procedure 2* is recommended.

Solution A: 0.1% Trifluoroacetic acid in water

Solution B: 0.1% Trifluoroacetic acid in acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
10.0	65	35
11.0	75	25
15.0	75	25

Diluent: Acetonitrile and water (75:25)

System suitability solution: 4 mg/mL of USP Metaxalone RS and 0.01 mg/mL of 3,5-dimethylphenol in *Diluent*

Sensitivity solution: 0.002 mg/mL of USP Metaxalone RS in *Diluent*

Sample solution: 4 mg/mL of Metaxalone in *Diluent*

Chromatographic system
(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 273 nm

Column: 4.6-mm × 25-cm; 5-μm packing L68

Flow rate: 2.0 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between metaxalone and 3,5-dimethylphenol, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Metaxalone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the peak responses of metaxalone and impurities from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Metaxalone	1.0	—
3,5-Dimethylphenol	1.1	0.05
Any individual unspecified impurity	—	0.05
Total impurities	—	0.50

• ORGANIC IMPURITIES, PROCEDURE 2

If metaxalone related compound B, metaxalone related compound C, or *N*-benzyl metaxalone is a known pro-

cess impurity, *Organic Impurities, Procedure 2* is recommended. If the article complies with *Procedure 2*, the labeling indicates that it meets *Organic Impurities, Procedure 2*.

Buffer, Mobile phase, and Standard stock solution: Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL of USP Metaxalone RS from the *Standard stock solution* in *Mobile phase*

Impurity stock solution: 0.2 mg/mL each of USP Metaxalone Related Compound B RS and USP Metaxalone Related Compound C RS in methanol. Sonicate to dissolve if necessary.

Peak identification solution: 1 mg/mL of USP Metaxalone RS and 0.02 mg/mL each of USP Metaxalone Related Compound B RS and USP Metaxalone Related Compound C RS prepared as follows. Transfer a suitable quantity of USP Metaxalone RS to a suitable volumetric flask. Add 50% of the flask volume of methanol, and sonicate to dissolve. Transfer suitable volumes of *Impurity stock solution* to the flask. Dilute with *Buffer* to volume.

Sample solution: 2.0 mg/mL of Metaxalone prepared as follows. Transfer a suitable quantity of Metaxalone to a suitable volumetric flask. Add 50% of the flask volume of methanol. Sonicate for 5 min to dissolve. Add 40% of the flask volume of *Buffer*, and mix. Cool to room temperature. Dilute with *Buffer* to volume.

Chromatographic system
(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 226 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: NLT 8 times the retention time of metaxalone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 10.0%

Signal-to-noise ratio: NLT 25

Analysis

Samples: *Standard solution* and *Sample solution*

Use the *Peak identification solution* to identify the peaks. Calculate the percentage of each impurity in the portion of Metaxalone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of metaxalone from the *Standard solution*

C_S = concentration of USP Metaxalone RS in the *Standard solution* (mg/mL)

C_U = concentration of Metaxalone in the *Sample solution* (mg/mL)

F = relative response factor for the corresponding impurity (see *Table 3*)

Acceptance criteria: See *Table 3*. Disregard any impurity peaks less than 0.03%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Metaxalone related compound B	0.35	1.0	0.05
Metaxalone	1.0	—	—

* 3-Benzyl-5-[(3,5-dimethylphenoxy)methyl]oxazolidin-2-one.

Table 3 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Metaxalone related compound C	3.6	1.0	0.05
N-Benzylmetaxalone ^a	6.9	0.64	0.05
Any individual unspecified impurity	—	1.0	0.05
Total impurities	—	—	0.3

^a 3-Benzyl-5-[(3,5-dimethylphenoxy)methyl]oxazolidin-2-one.

SPECIFIC TESTS• **LOSS ON DRYING** (731)

Analysis: Dry at 90° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The label states with which *Organic Impurities* procedure the article complies if *Organic Impurities, Procedure 1* is not used.
- **USP REFERENCE STANDARDS** (11)
 - USP Metaxalone RS
 - USP Metaxalone Related Compound B RS
 - 1-Amino-3-(3,5-dimethylphenoxy)propan-2-ol.
C₁₁H₁₇NO₂ 195.26
 - USP Metaxalone Related Compound C RS
 - Bis[2-hydroxy-3-(3,5-dimethylphenoxy)propyl]amine.
C₂₂H₃₁NO₄ 373.49

Metaxalone Tablets**DEFINITION**

Metaxalone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metaxalone (C₁₂H₁₅NO₃).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Buffer: 0.68 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 4.5.

Mobile phase: Methanol and *Buffer* (50:50)

Standard stock solution: 0.5 mg/mL of USP Metaxalone RS prepared as follows. Transfer a suitable amount of USP Metaxalone RS to a suitable volumetric flask. Add 50% of the flask volume of methanol and sonicate to dissolve. Dilute with *Buffer* to volume.

Standard solution: 0.05 mg/mL of USP Metaxalone RS from *Standard stock solution* in *Mobile phase*.

Sample stock solution: Nominally 1.0 mg/mL of metaxalone from NLT 20 Tablets prepared as follows. Transfer a portion of finely powdered Tablets equivalent to NLT 500 mg of metaxalone to a suitable volumetric flask. Add 50% of the flask volume of methanol and sonicate for 10 min with occasional swirling. Shake on a mechanical shaker for 15 min. Add 40% of the flask volume of *Buffer* and allow the solution to cool to room temperature. Dilute with *Buffer* to volume. Pass a portion of the solution through a PVDF filter of 0.45-μm pore size. Discard the first 5 mL. Use the filtrate.

Sample solution: Nominally 0.05 mg/mL of metaxalone from *Sample stock solution* and *Mobile phase*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 226 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: NLT 2 times the retention time of metaxalone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metaxalone (C₁₂H₁₅NO₃) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metaxalone from the *Sample solution*

r_S = peak response of metaxalone from the *Standard solution*

C_S = concentration of USP Metaxalone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metaxalone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)

Medium: 0.5% sodium lauryl sulfate; 900 mL

Apparatus 2: 100 rpm

Time: 60 min

Buffer, Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay*, except use 270 nm for analysis.

Standard solution: (L/900) mg/mL of USP Metaxalone RS, where L is the label claim of metaxalone, in mg/ Tablet, prepared as follows. Transfer a suitable quantity of USP Metaxalone RS to a suitable volumetric flask. Add 4% of the flask volume of methanol, sonicate to dissolve, and dilute with *Medium* to volume.

Sample solution: Pass a portion of the solution under test through a suitable PVDF membrane filter of 0.45-μm pore size. Discard the first 5 mL of the filtrate and use the remaining amount for analysis.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metaxalone (C₁₂H₁₅NO₃) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Metaxalone RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim of metaxalone (mg/Tablet)

Tolerances: NLT 60% (Q) of the labeled amount of metaxalone (C₁₂H₁₅NO₃) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, Standard solution, and Chromatographic system: Proceed as directed in the *Assay*.

Impurity stock solution: 0.2 mg/mL each of USP Metaxalone Related Compound B RS and USP Metax-

alone Related Compound C RS in methanol. Sonicate to dissolve if necessary.

Peak identification solution: 1 mg/mL of USP Metaxalone RS and 0.02 mg/mL each of USP Metaxalone Related Compound B RS and USP Metaxalone Related Compound C RS prepared as follows. Transfer a suitable quantity of USP Metaxalone RS to a suitable volumetric flask. Add 50% of the flask volume of methanol and sonicate to dissolve. Transfer suitable volumes of *Impurity stock solution* to the flask. Dilute with *Buffer* to volume.

Sensitivity solution: 0.5 µg/mL of USP Metaxalone RS from *Standard solution* and *Mobile phase*

Sample solution: Nominally 1.0 mg/mL of metaxalone prepared from NLT 20 Tablets as follows. Transfer a portion of NLT 20 finely powdered Tablets equivalent to NLT 500 mg of metaxalone to a suitable volumetric flask. Add 50% of the flask volume of methanol and sonicate for 10 min with occasional swirling. Shake on a mechanical shaker for 15 min. Add 40% of the flask volume of *Buffer* and cool to room temperature. Dilute with *Buffer* to volume. Pass a portion of the solution through a PVDF filter of 0.45-µm pore size. Discard the first 5 mL.

System suitability

Samples: *Peak identification solution* and *Sensitivity solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Tailing factor: NMT 2.0, *Sensitivity solution*

Relative standard deviation: NMT 10.0% for the metaxalone peak, *Sensitivity solution*

Signal-to-noise ratio: NLT 25 for the metaxalone peak, *Sensitivity solution*

Analysis

Samples: *Standard solution*, *Peak identification solution*, and *Sample solution*

Use the *Peak identification solution* to identify the peaks. Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each degradation product from the *Sample solution*

r_S = peak response of metaxalone from the *Standard solution*

C_S = concentration of USP Metaxalone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metaxalone in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Metaxalone related compound B	0.35	0.15
Metaxalone	1.0	—
Metaxalone related compound C ^a	3.6	—
N-Benzylmetaxalone ^b	6.9	—

^a Process impurity, included for peak identification only; monitored in the drug substance.

^b 3-Benzyl-5-[(3,5-dimethylphenoxy)methyl]oxazolidin-2-one.

Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified degradation product	—	0.10
Total degradation products	—	0.5

^a Process impurity, included for peak identification only; monitored in the drug substance.

^b 3-Benzyl-5-[(3,5-dimethylphenoxy)methyl]oxazolidin-2-one.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Metaxalone RS

USP Metaxalone Related Compound B RS

1-Amino-3-(3,5-dimethylphenoxy)propan-2-ol.

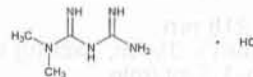
$C_{11}H_{17}NO_2$ 195.26

USP Metaxalone Related Compound C RS

Bis[2-hydroxy-3-(3,5-dimethylphenoxy)propyl]amine.

$C_{22}H_{31}NO_4$ 373.49

Metformin Hydrochloride



$C_4H_{11}N_5 \cdot HCl$ 165.62
Imidodicarbonimidic diamide, *N,N*-dimethyl-, monohydrochloride;
1,1-Dimethylbiguanide monohydrochloride [1115-70-4].

DEFINITION

Metformin Hydrochloride contains NLT 98.5% and NMT 101.0% of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION (197K)**

• **B. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements

ASSAY

• PROCEDURE

Sample: 60 mg of Metformin Hydrochloride

Analysis

[NOTE—To avoid overheating of the reaction medium, mix thoroughly throughout the titration, and stop the titration immediately after the endpoint has been reached.]

Dissolve the *Sample* in 4 mL of anhydrous formic acid, and add 50 mL of acetic anhydride. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 8.28 mg of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$).

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method I** (231): NMT 10 ppm (Official).

(Jan-2018)

ORGANIC IMPURITIES

Mobile phase: 17 g/L of monobasic ammonium phosphate in water, adjusted with phosphoric acid to a pH of 3.0

System suitability stock solution: 0.25 mg/mL of metformin hydrochloride and 0.1 mg/mL of melamine in water

System suitability solution: Transfer 1.0 mL of the System suitability stock solution to a 50-mL volumetric flask, and dilute with Mobile phase to volume.

Standard stock solution: 0.2 mg/mL of USP Metformin Related Compound A RS in water

Standard solution: 0.001 mg/mL of USP Metformin Related Compound A RS in Mobile phase from the Standard stock solution

Sample solution: 5 mg/mL of Metformin Hydrochloride in Mobile phase

Diluted sample solution: 0.005 mg/mL of Metformin Hydrochloride in Mobile phase from the Sample solution

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 218 nm

Column: 4.6-mm × 25-cm; packing L9

Flow rate: 1.0–1.7 mL/min

Run time: NLT twice the retention time of metformin

Injection volume: 20 µL

System suitability

Sample: System suitability solution

Suitability requirements

Resolution: NLT 10 between melamine and metformin

Analysis

Samples: Standard solution, Sample solution, and Diluted sample solution

Calculate the percentage of metformin related compound A in the portion of Metformin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metformin related compound A from the Sample solution

r_S = peak response of metformin related compound A from the Standard solution

C_S = concentration of USP Metformin Related Compound A RS in the Standard solution (mg/mL)

C_U = concentration of Metformin Hydrochloride in the Sample solution (mg/mL)

Calculate the percentage of any other impurity in the portion of Metformin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

r_U = peak response of an individual impurity from the Sample solution

r_S = peak response of metformin from the Diluted sample solution

D = dilution factor for the preparation of the Diluted sample solution, 0.001

Acceptance criteria

Individual impurities: NMT 0.02% for metformin related compound A; NMT 0.1% for any other impurity

Total impurities: NMT 0.5%

SPECIFIC TESTS

- **LOSS ON DRYING** (731)

Analysis: Dry a sample at 105° for 5 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

- **USP REFERENCE STANDARDS** (11)

USP Metformin Hydrochloride RS

USP Metformin Related Compound A RS

1-Cyanoguanidine.

$C_2H_4N_4$ 84.08

Metformin Hydrochloride Tablets

DEFINITION

Metformin Hydrochloride Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

Sample: Transfer an amount of powdered Tablets, equivalent to 20 mg of metformin hydrochloride, to a suitable flask. Add 20 mL of dehydrated alcohol, and shake. Filter, evaporate the filtrate on a water bath to dryness, and dry the residue at 105° for 2 h.

Acceptance criteria: Meet the requirements

- **B.**

Solution A: Dissolve 1 g of 1-naphthol in a solution containing 6 g of sodium hydroxide and 16 g of anhydrous sodium carbonate in 100 mL of water.

Sample solution: Triturate an amount of powdered Tablets, equivalent of 50 mg of metformin hydrochloride, with 10 mL of water, filter, and use the filtrate.

Analysis: To 5 mL of the Sample solution add 1.5 mL of 5 N sodium hydroxide solution and 1 mL of Solution A. Add 0.5 mL of sodium hypochlorite TS, dropwise, and with shaking.

Acceptance criteria: An orange-red color is produced that darkens on standing.

- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191)

Sample solution: Prepare as directed for the Sample solution in Identification test B.

Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**

Standard solution: 10 µg/mL of USP Metformin Hydrochloride RS in water

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer the amount of powder, equivalent to 100 mg of metformin hydrochloride, to a 100-mL volumetric flask. Add 70 mL of water, shake by mechanical means for 15 min, dilute with water to volume, and filter, discarding the first 20 mL of the filtrate. Dilute 10.0 mL of the filtrate with water to 100.0 mL, and dilute 10.0 mL of the resulting solution with water to 100.0 mL. The nominal concentration of this solution is 10 µg/mL.

Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

Mode: UV

Analytical wavelength: Wavelength of maximum absorbance at about 232 nm

Cell: 1 cm
Blank: Water

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the portion of the Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* ($\mu\text{g/mL}$)
 C_U = nominal concentration of metformin hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)
Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Medium: pH 6.8 phosphate buffer; 1000 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: USP Metformin Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: Wavelength of maximum absorbance at about 233 nm

Analysis: Determine the amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved by using UV absorption of filtered portions of the *Sample solution*, suitably diluted with *Medium*, if necessary, in comparison with the *Standard solution*.

Tolerances: NLT 70% (Q) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

For products labeled to contain 500 mg of metformin hydrochloride

Medium: pH 6.8 phosphate buffer; 1000 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution, Sample solution, Instrumental conditions, and Analysis: Proceed as directed in *Test 1*.

Tolerances: NLT 80% (Q) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.

For products labeled to contain 850 or 1000 mg of metformin hydrochloride

Medium: pH 6.8 phosphate buffer; 1000 mL

Apparatus 2: 75 rpm

Time: 30 min

Standard solution, Sample solution, Instrumental conditions, and Analysis: Proceed as directed in *Test 1*.

Tolerances: NLT 75% (Q) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: pH 6.8 phosphate buffer; 1000 mL

Apparatus 1: 100 rpm

Time: 60 min

Buffer: Dissolve 1.38 g of monobasic sodium phosphate in about 1800 mL of water. Add 3.484 g of 1-pentanesulfonic acid sodium salt. Adjust with diluted phosphoric acid to a pH of 3.00 ± 0.05 . Dilute with water to 2000 mL.

Mobile phase: Acetonitrile and *Buffer* (1:19)

Standard stock solution: 0.25 mg/mL of USP

Metformin Hydrochloride RS in *Medium*. Use sonication to dissolve.

Standard solution: 0.05 mg/mL of USP Metformin Hydrochloride RS in *Medium* from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a nylon filter of 0.45- μm pore size. Dilute with *Medium*, if necessary, to obtain a solution with a concentration similar to that of the *Standard solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L1

Flow rate: 1.0 mL/min

Injection volume: 40 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 1500 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (V/D) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 1000 mL

D = dilution factor of the *Sample solution*

Tolerances: NLT 70% (Q) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase: 17 g/L of monobasic ammonium phosphate in water, adjusted with phosphoric acid to a pH of 3.0

System suitability stock solution: 0.25 mg/mL of metformin hydrochloride and 0.1 mg/mL of melamine in water

System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer the amount of powder, equivalent to 500 mg of metformin hydrochloride, to a 100-mL volumetric flask. Dissolve in *Mobile phase* with shaking, dilute with *Mobile phase* to volume, and filter.

Diluted sample solution: Nominally 0.005 mg/mL of metformin hydrochloride in *Mobile phase* from the *Sample solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 218 nm

Column: 4.6-mm \times 25-cm; packing L9

Flow rate: 1.0–1.7 mL/min

Run time: NLT twice the retention time of metformin

Injection volume: 20 μL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 10 between melamine and metformin

Analysis

Samples: *Sample solution* and *Diluted sample solution*
Calculate the percentage of any individual impurity in the portion of the Tablets taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

- r_U = peak response of any individual impurity from the *Sample solution*
 r_S = peak response of metformin from the *Diluted sample solution*
 D = dilution factor for the preparation of the *Diluted sample solution*, 0.001

Acceptance criteria

Any individual impurity: NMT 0.1%
 Total impurities: NMT 0.6%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
USP Metformin Hydrochloride RS

Metformin Hydrochloride Extended-Release Tablets

DEFINITION

Metformin Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Buffer solution: 0.5 g/L of sodium 1-heptanesulfonate and 0.5 g/L of sodium chloride in water. Before final dilution, adjust with 0.06 M phosphoric acid to a pH of 3.85.

Mobile phase: Acetonitrile and *Buffer solution* (1:9).

[NOTE—To improve the separation, the composition of acetonitrile and *Buffer solution* may be changed to 1:19, if necessary.]

Diluent: 1.25% solution of acetonitrile in water

Standard solution: ($L/4000$) mg/mL of USP Metformin Hydrochloride RS in *Diluent*, where L is the labeled quantity, in mg, of metformin hydrochloride in each Tablet

System suitability stock solution: 12.5 µg/mL each of USP Metformin Related Compound B RS and USP Metformin Related Compound C RS in *Diluent*

System suitability solution: Dilute 0.5 mL of the *System suitability stock solution* with the *Standard solution* to 50 mL.

Sample stock solution: Finely powder NLT 10 Tablets. Transfer powder, equivalent to the average Tablet weight, to a homogenization vessel, and add 500 mL of a 10% acetonitrile solution. Alternately, homogenize and allow to soak until the sample is fully homogenized. [NOTE—A suggested homogenization sequence is as follows. Homogenize the sample using five pulses, each of 5 s, at about 20,000 rpm, and allow to soak for 2 min. Repeat these steps two additional times.]

Sample solution: Pass a portion of the *Sample stock solution* through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate. Transfer 25 mL of

the filtrate to a 200-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 218 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: Until after the elution locus of metformin related compound C

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for metformin related compound B, metformin, and metformin related compound C are 0.86, 1.0, and 2.1–2.3, respectively. Metformin related compound C can have a variable retention time. The composition of the *Mobile phase* may be changed to 1:19, if it elutes at a relative retention time of less than 2.1.]

Suitability requirements

Resolution: NLT 1.5 between the peaks due to metformin related compound B and metformin

Tailing factor: NLT 0.8 and NMT 2.0 for the metformin peak

Relative standard deviation: NMT 1.5% for the metformin peak and NMT 10% for each of the peaks due to metformin related compound B and metformin related compound C

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metformin hydrochloride in the *Sample solution*

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS**Change to read:****• DISSOLUTION (711)****Test 1**

Medium: pH 6.8 phosphate buffer solution; 1000 mL

Apparatus 1: 100 rpm for Tablets labeled to contain 750 mg

Apparatus 2: 100 rpm for Tablets labeled to contain 500 mg

Times: 1, 3, and 10 h

Detector: UV 232 nm

Standard solution: USP Metformin Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable hydrophilic polyethylene filter of 0.45-µm pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.

Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) released at each time point:

$$\text{Result} = [(A_U/A_S) \times C_S \times (V - V_S) + (C_{60} \times V_S) + (C_{180} \times V_S)] \times (100/L)$$

A_U = absorbance of the *Sample solution*

- A_s = absorbance of the *Standard solution*
 C_s = concentration of the *Standard solution* (mg/mL)
 V = initial volume of *Medium* in the vessel (mL)
 V_s = volume withdrawn from the vessel for previous samplings (mL)
 C_{60} = concentration of metformin hydrochloride in *Medium* determined at 1 h (mg/mL)
 C_{180} = concentration of metformin hydrochloride in *Medium* determined at 3 h (mg/mL)
 L = label claim (mg/Tablet)
Tolerances: See Table 1.

Table 1

Time (h)	Amount Dissolved, 500-mg Tablet (%)	Amount Dissolved, 750-mg Tablet (%)
1	20–40	22–42
3	45–65	49–69
10	NLT 85	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Prepare as directed for *Test 1*; 1000 mL.

Apparatus 2: 100 rpm

Times: 1, 2, 6, and 10 h

Detector: UV 232 nm

Standard solution: USP Metformin Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable polyethylene filter of 0.45- μ m pore size. Dilute, if necessary, with *Medium* to a concentration that is similar to that of the *Standard solution*.

Analysis: Calculate, in mg/mL, the content of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), C_u , in *Medium* at each time point, t :

$$\text{Result} = (A_u \times C_s \times D_u) / A_s$$

- A_u = absorbance of the *Sample solution*
 C_s = concentration of metformin hydrochloride in the *Standard solution* (mg/mL)
 D_u = dilution factor of the solution under test
 A_s = absorbance of the *Standard solution*

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at each time point by the following formulas.

Percentage dissolved at the first time point (1 h):

$$\text{Result} = (C_1 \times V \times 100) / L$$

- C_1 = content of metformin hydrochloride in *Medium* at the first time interval (mg/mL)
 V = volume of *Medium*, 1000 mL
 L = label claim (mg/Tablet)

Percentage dissolved at the second time point (2 h):

$$\text{Result} = [C_2 \times (V - SV_1) + C_1 \times SV_1] \times (100/L)$$

- C_2 = content of metformin hydrochloride in *Medium* at the second time interval (mg/mL)
 V = volume of *Medium*, 1000 mL
 SV_1 = volume of the sample withdrawn at 1 h (mL)
 C_1 = content of metformin hydrochloride in *Medium* at 1 h (mg/mL)
 L = label claim (mg/Tablet)

Percentage dissolved at the n th time point:

$$\text{Result} = \{C_n \times [V - (n - 1)V_s] + (C_1 + C_2 + \dots + C_{n-1}) \times V_s\} \times (100/L)$$

- C_n = content of metformin hydrochloride in *Medium* at the n th time interval (mg/mL)
 V = volume of *Medium*, 1000 mL
 n = time interval of interest
 V_s = volume of sample withdrawn at each time interval (mL)
 C = as $C_1, C_2, C_3, \dots, C_{n-1}$, the content of metformin hydrochloride in *Medium* at each time interval (mg/mL)
 L = label claim (mg/Tablet)
Tolerances: See Table 2.

Table 2

Time (h)	Amount Dissolved (%)
1	20–40
2	35–55
6	65–85
10	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium, Apparatus 1, and Apparatus 2 • (ERR 1–Jun-2016): Proceed as directed in *Test 1*.

Times: 1, 2, 5, and 12 h for Tablets labeled to contain 500 mg; and 1, 3, and 10 h for Tablets labeled to contain 750 mg

Detector: UV 232 nm

Standard solution: USP Metformin Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable hydrophilic polyethylene filter of 0.45- μ m pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.

Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) released at each time point:

$$\text{Result} = \{[(A_u/A_s) \times C_s \times (V - V_s) + (C_{60} \times V_s) + (C_{120} \times V_s) + (C_{300} \times V_s) + (C_{720} \times V_s)] \times 100\} / L$$

- A_u = absorbance of the *Sample solution*
 A_s = absorbance of the *Standard solution*
 C_s = concentration of the *Standard solution* (mg/mL)
 V = initial volume of *Medium* in the vessel (mL)
 V_s = volume withdrawn from the vessel for previous samplings (mL)
 C_{60} = concentration of metformin hydrochloride in *Medium* determined at 1 h (mg/mL)
 C_{120} = concentration of metformin hydrochloride in *Medium* determined at 2 h (mg/mL)
 C_{300} = concentration of metformin hydrochloride in *Medium* determined at 5 h (mg/mL)
 C_{720} = concentration of metformin hydrochloride in *Medium* determined at 12 h (mg/mL)
 L = label claim (mg/Tablet)

Tolerances: See Tables 3 and 4.

Table 3. For Tablets Labeled to Contain 500 mg

Time (h)	Amount Dissolved (%)
1	20–40
2	35–55
5	60–80
12	NLT 85

Table 4. For Tablets Labeled to Contain 750 mg

Time (h)	Amount Dissolved (%)
1	22–42
3	49–69
10	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: Prepare as directed for *Test 1*; 1000 mL.

Apparatus 2: 100 rpm

Times: 1, 3, 6, and 10 h

Detector: UV 250 nm (shoulder)

Standard solution: USP Metformin Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.

Analysis: Calculate, in mg/mL, the content of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), C_t , in *Medium* at each time point, t , by the formulas specified in *Test 2*.

Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved (%)
1	20–40
3	45–65
6	65–85
10	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium: pH 6.8 phosphate buffer solution; 900 mL, deaerated

Apparatus 1: 100 rpm, with the vertical holder described in *Figure 1* and *Figure 2*

Times: 2, 8, and 16 h

Detector: UV 250 nm

Standard solution: USP Metformin Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.

Analysis: Place a vertical sample holder into each basket (see *Figures 1* and *2*). Place 1 Tablet inside the sample holder, making sure that the Tablets are vertical at the bottom of the baskets.

Calculate, in mg/mL, the content of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$), C_t , in *Medium* at each time point, t , by the formulas specified in *Test 2*.

Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Dissolved, 500-mg Tablet (%)	Amount Dissolved, 1000-mg Tablet (%)
2	NMT 30	NMT 30
8	60–85	65–90
16	NLT 90	NLT 90

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium: pH 6.8 phosphate buffer solution; 1000 mL, deaerated

Apparatus 2: 100 rpm, with USP sinker, if necessary

Detector: UV 233 nm

Standard solution: USP Metformin Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable hydrophilic polyethylene filter of 0.45- μ m pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.

Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) released at each time point:

$$\text{Result} = \left[\frac{(A_U/A_S) \times C_S \times (V - V_S) + (C_{60} \times V_S) + (C_{180} \times V_S) + (C_{600} \times V_S)}{V_S} \right] \times 100 / L$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = initial volume of *Medium* in the vessel (mL)

V_S = volume withdrawn from the vessel for previous samplings (mL)

C_{60} = concentration of metformin hydrochloride in *Medium* determined at 1 h (mg/mL)

C_{180} = concentration of metformin hydrochloride in *Medium* determined at 3 h (mg/mL)

C_{600} = concentration of metformin hydrochloride in *Medium* determined at 10 h (mg/mL)

L = label claim (mg/Tablet)

Tolerances: See *Table 7*.

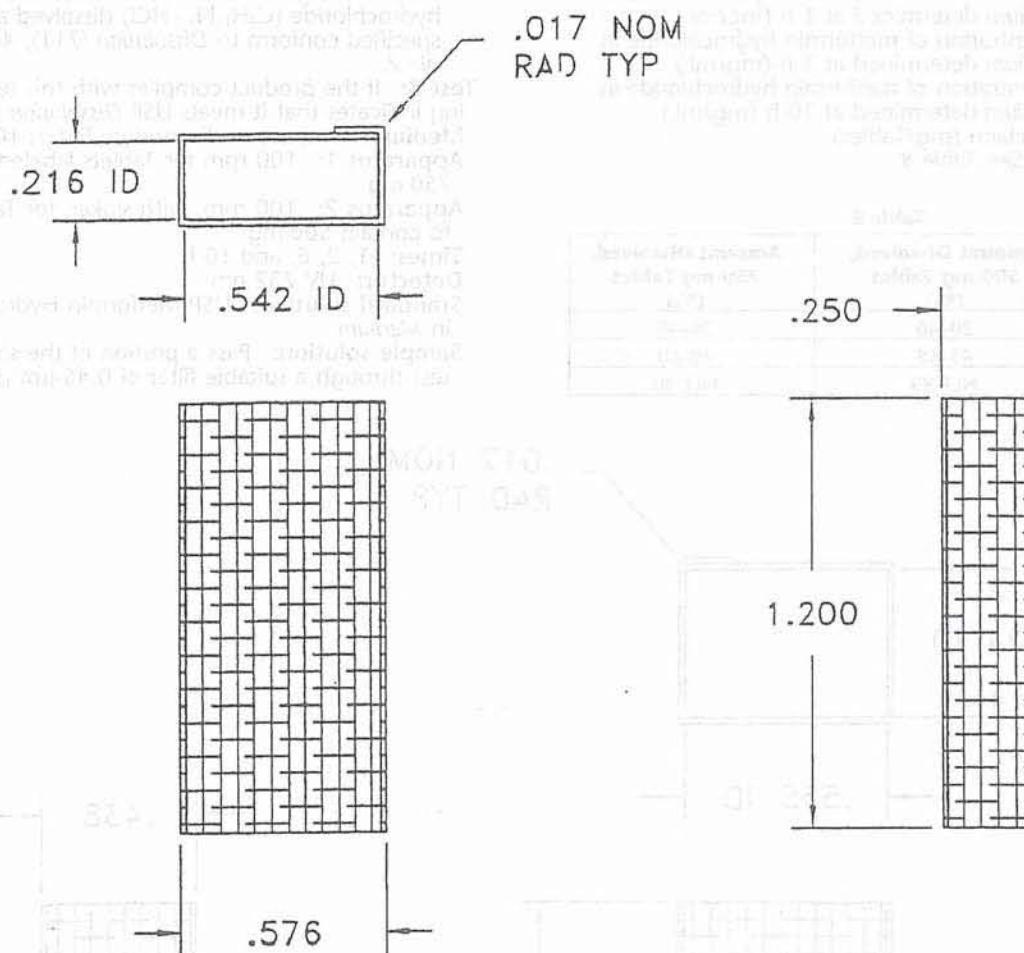
Table 7

Time (h)	Amount Dissolved, 500-mg Tablet (%)	Amount Dissolved, 750-mg Tablet (%)
1	20–40	20–40
3	45–65	45–65
10	NLT 85	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

Medium: Prepare as directed in *Test 1*; 1000 mL.



NOTES:

1. MATERIAL: 316SS OR EQUIVALENT .017 WIRE VERTICAL MEAS SQUARE WEAVE WITH .039 SQUARE OPENINGS.
2. ALL DIMENSIONS ARE IN INCHES. TOLERANCES TO BE +/- .010

Figure 1

Apparatus 1: 100 rpm for Tablets labeled to contain 750 mg

Apparatus 2: 50 rpm, with USP sinker, for Tablets labeled to contain 500 mg

Times: 1, 3, and 10 h

Detector: UV 232 nm

Standard solution: USP Metformin Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size. Dilute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.

Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) released at each time point:

$$\text{Result} = \left\{ \left[\frac{(A_U/A_S) \times C_S \times (V - V_S) + (C_{60} \times V_S) + (C_{180} \times V_S) + (C_{600} \times V_S)}{V_S} \right] \times 100 \right\} / L$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = initial volume of *Medium* in the vessel (mL)

V_S = volume withdrawn from the vessel for previous samplings (mL)

- C_{60} = concentration of metformin hydrochloride in Medium determined at 1 h (mg/mL)
 C_{180} = concentration of metformin hydrochloride in Medium determined at 3 h (mg/mL)
 C_{600} = concentration of metformin hydrochloride in Medium determined at 10 h (mg/mL)
 L = label claim (mg/Tablet)
 Tolerances: See Table 8.

Table 8

Time (h)	Amount Dissolved, 500-mg Tablet (%)	Amount Dissolved, 750-mg Tablet (%)
1	20–40	20–40
3	45–65	40–60
10	NLT 85	NLT 80

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 8: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

Medium: Prepare as directed in *Test 1*; 1000 mL.

Apparatus 1: 100 rpm for Tablets labeled to contain 750 mg

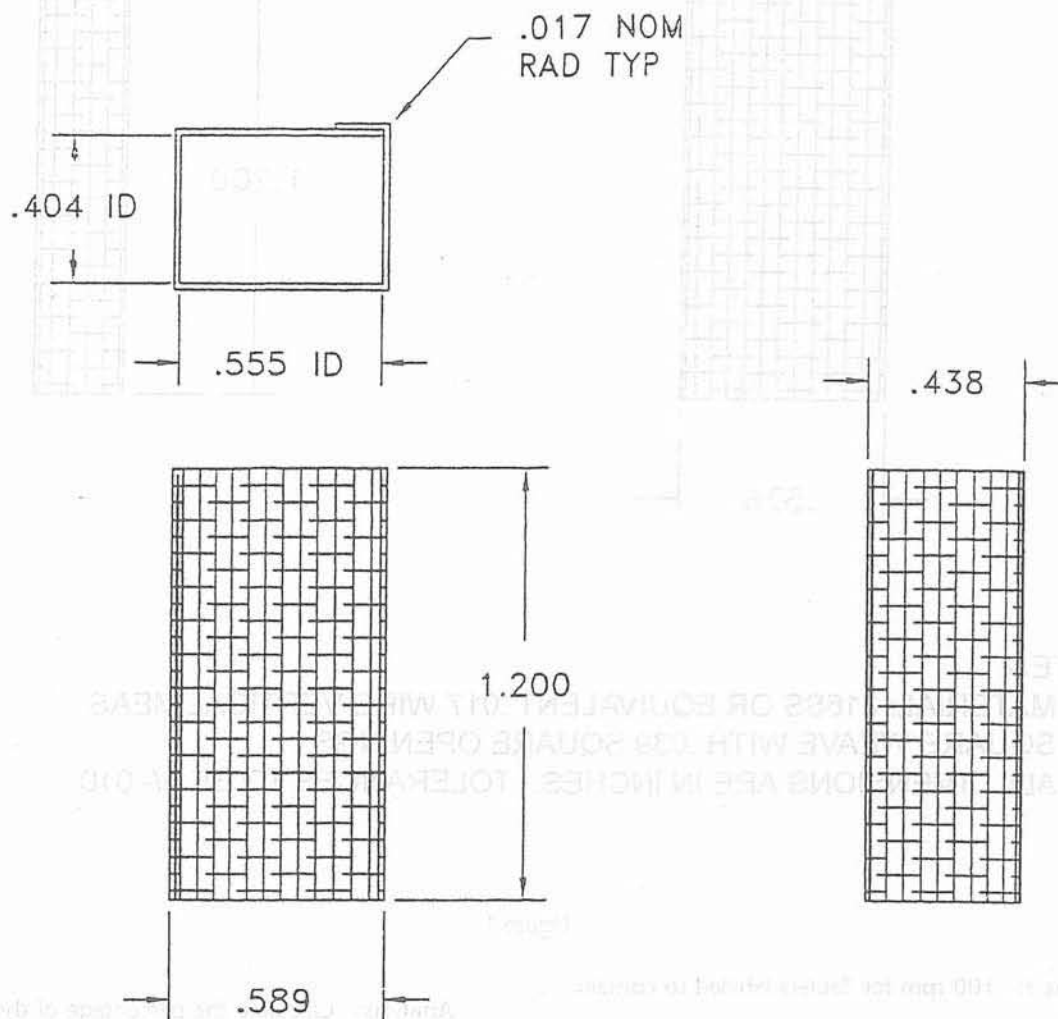
Apparatus 2: 100 rpm, with sinker, for Tablets labeled to contain 500 mg

Times: 1, 2, 6, and 10 h

Detector: UV 232 nm

Standard solution: USP Metformin Hydrochloride RS in Medium

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size. Di-



NOTES:

1. MATERIAL: 316SS OR EQUIVALENT .017 WIRE VERTICAL MEAS SQUARE WEAVE WITH .039 SQUARE OPENINGS.
2. ALL DIMENSIONS ARE IN INCHES. TOLERANCES TO BE $\pm .010$

Figure 2

lute, if necessary, with *Medium* to a concentration similar to that of the *Standard solution*.

Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) released at each time point:

$$\text{Result} = \frac{[(A_U/A_S) \times C_S \times (V - V_S) + (C_{60} \times V_S) + (C_{120} \times V_S) + (C_{360} \times V_S) + (C_{600} \times V_S)] \times 100}{L}$$

- A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)
 V = initial volume of *Medium* in the vessel (mL)
 V_S = volume withdrawn from the vessel for previous samplings (mL)
 C_{60} = concentration of metformin hydrochloride in *Medium* determined at 1 h (mg/mL)
 C_{120} = concentration of metformin hydrochloride in *Medium* determined at 2 h (mg/mL)
 C_{360} = concentration of metformin hydrochloride in *Medium* determined at 6 h (mg/mL)
 C_{600} = concentration of metformin hydrochloride in *Medium* determined at 10 h (mg/mL)
 L = label claim (mg/Tablet)

Tolerances: See Table 9.

Table 9

Time (h)	Amount Dissolved, 500-mg Tablet (%)	Amount Dissolved, 750-mg Tablet (%)
1	20–40	20–40
2	30–50	35–55
6	65–85	75–95
10	NLT 85	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 9: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.

Medium: 0.05 M phosphate buffer, pH 6.8; 1000 mL

Apparatus 1: 100 rpm, for Tablets labeled to contain 750 mg

Apparatus 2: 100 rpm, for Tablets labeled to contain 500 mg

Times: 1, 5, 12, and 20 h for Tablets labeled to contain 500 mg; and 1, 4, 10, and 24 h for Tablets labeled to contain 750 mg

Standard solution: 0.5 mg/mL of USP Metformin Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Detector: UV 232 nm

Path length: 0.01 cm, flow cell

Blank: *Medium*

Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) released at each time point:

$$\text{Result} = \frac{[(A_U/A_S) \times C_S \times (V - V_S) + (C_1 \times V_S) + (C_2 \times V_S) + (C_3 \times V_S) + (C_4 \times V_S)] \times 100}{L}$$

- A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)
 V = initial volume of *Medium* in the vessel (mL)
 V_S = volume withdrawn from the vessel for previous samplings (mL)
 C_1 = concentration of metformin hydrochloride in *Medium* determined at the first time point (mg/mL)

C_2 = concentration of metformin hydrochloride in *Medium* determined at the second time point (mg/mL)

C_3 = concentration of metformin hydrochloride in *Medium* determined at the third time point (mg/mL)

C_4 = concentration of metformin hydrochloride in *Medium* determined at the fourth time point (mg/mL)

L = label claim (mg/Tablet)

Tolerances: See Tables 10 and 11.

Table 10. For Tablets Labeled to Contain 500 mg

Time (h)	Amount Dissolved (%)
1	20–40
5	45–65
12	70–90
20	NLT 85

Table 11. For Tablets Labeled to Contain 750 mg

Time (h)	Amount Dissolved (%)
1	20–45
4	45–70
10	70–95
24	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 10: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 10*.
Medium: 0.05 M phosphate buffer (prepared by dissolving 6.8 g of monobasic potassium phosphate in 250 mL of water, adding 77 mL of 0.2 N sodium hydroxide and 500 mL of water, adjusting with 2 N sodium hydroxide or 2 N hydrochloric acid to a pH 6.8, and diluting with water to 1000 mL)

Apparatus 1: 100 rpm for Tablets labeled to contain 750 mg

Apparatus 2: 100 rpm for Tablets labeled to contain 500 mg

Times: 1, 3, and 10 h

Standard solution: ($L/100,000$) mg/mL of USP Metformin Hydrochloride RS in *Medium*, where L is the label claim, in mg/Tablet. This solution is stable for 72 h at room temperature.

Sample solution: At the times specified, withdraw 10 mL of the solution under test and replace with 10 mL of *Medium* previously equilibrated at $37.0 \pm 0.5^\circ$. Centrifuge at 2500 rpm for 10 min. Dilute a portion of the supernatant with *Medium* to obtain a theoretical concentration of ($L/100,000$) mg/mL, where L is the label claim, in mg/Tablet.

Detector: UV 233 nm

Path length: 1 cm

Blank: *Medium*

Analysis: Calculate the concentration (mg/mL) of metformin hydrochloride (C) at each time point:

$$C_i = (A_U/A_S) \times C_S$$

- A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)

Calculate the cumulative percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved (Q_i) at each time point (i):

At $i = 1$:

$$Q_1 = (C_1 \times V/L) \times 100$$

At $i = 3$:

$$Q_3 = [C_3(V - V_3) + (C_1 \times V_3)] \times 100/L$$

At $i = 10$:

$$Q_{10} = [C_{10}(V - 2V_3) + (C_1 + C_3)V_3] \times 100/L$$

V = initial volume of *Medium*, 1000 mL

V_3 = sampling volume, 10 mL

L = label claim (mg/Tablet)

Tolerances: See Table 12.

Table 12

Time (h)	Amount Dissolved (%)
1	25–45
3	50–70
10	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 11: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 11*.

Medium: pH 6.8 phosphate buffer solution; 1000 mL

Apparatus 1: 100 rpm for Tablets labeled to contain 750 mg

Apparatus 2: 100 rpm for Tablets labeled to contain 500 mg

Times: 1, 3, and 10 h

Standard solution: 7.5 µg/mL of USP Metformin Hydrochloride RS in *Medium*

Sample solution: At the times specified, withdraw 10 mL of the solution under test, and pass it through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate. Dilute 3.0 mL of the filtrate with *Medium* to 200 mL. For Tablets labeled to contain 750 mg, dilute 2.0 mL of the filtrate with *Medium* to 200 mL. Replace the volume of *Medium* taken with the same volume of *Medium* preheated at $37.0 \pm 0.5^\circ$.

Detector: UV 232 nm

Path length: 1 cm

Blank: *Medium*

Analysis: Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at each time point:

$$Q_i = (A_u/A_s) \times (C_s/L) \times V \times D \times 100$$

At 1 h:

$$\text{Result} = Q_1$$

At 3 h:

$$\text{Result} = Q_3 + [(Q_1 \times 10)/V]$$

At 10 h:

$$\text{Result} = Q_{10} + [(Q_1 \times 10)/V] + [(Q_3 \times 10)/V]$$

A_u = absorbance of the *Sample solution*

A_s = absorbance of the *Standard solution*

C_s = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 1000 mL

D = dilution factor of the *Sample solution*

Tolerances: See Table 13.

Table 13

Time (h)	Amount Dissolved (%)
1	25–45
3	50–70
10	NLT 80

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 12: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 12*.

Medium: pH 6.8 phosphate buffer solution; 1000 mL

Apparatus 1: 100 rpm

Times: 1, 4, and 12 h

Standard stock solution: 0.2 mg/mL of USP Metformin Hydrochloride RS in *Medium*

Standard solution: 0.01 mg/mL of USP Metformin Hydrochloride RS in water, from the *Standard stock solution*

Sample solution: At the times specified, withdraw 10 mL of the solution under test, and replace with 10 mL of *Medium* previously equilibrated at $37.0 \pm 0.5^\circ$. Pass it through a suitable filter, discarding the first few mL of the filtrate.

For Tablets labeled to contain 500 mg: Dilute 2.0 mL of the filtrate with water to 100 mL.

For Tablets labeled to contain 1000 mg: Dilute 1.0 mL of the filtrate with water to 100 mL.

Detector: UV 232 nm

Blank: Dilute 1 mL of *Medium* with water to 100 mL.

Analysis: Calculate the concentration, C_i , in mg/mL of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the sample withdrawn at each time point (i):

$$\text{Result}_i = (A_u/A_s) \times C_s \times D$$

A_u = absorbance of the *Sample solution*

A_s = absorbance of the *Standard solution*

C_s = concentration of the *Standard solution* (mg/mL)

D = dilution factor of the *Sample solution*

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved (Q_i) at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = [(C_3 \times V) + [(C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

C_i = concentration of metformin hydrochloride in the portion of sample withdrawn at time point i (mg/mL)

V = initial volume of *Medium*, 1000 mL

L = label claim (mg/Tablet)

V_3 = volume of the *Sample solution* withdrawn, 10 mL

Tolerances: See Table 14.

Table 14

Time point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 15
2	4	35–65
3	12	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 13: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 13*.
Medium: pH 6.8 phosphate buffer solution; 1000 mL
Apparatus 1: 100 rpm

Times: 1, 4, 6, and 14 h

Standard stock solution: 0.2 mg/mL of USP

Metformin Hydrochloride RS prepared as follows.

Transfer a suitable amount of USP Metformin Hydrochloride RS into an appropriate volumetric flask. Dissolve by adding *Medium* to fill 50% of the flask volume and dilute with *Medium* to volume.

Standard solution: 0.01 mg/mL of USP Metformin Hydrochloride RS from *Standard stock solution* in water

Sample stock solution: At the times specified, withdraw 10 mL of the solution under test, and replace with the same volume of *Medium* preheated at $37.0 \pm 0.5^\circ$. Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size, discard the first few mL, and use the filtrate.

Sample solution

For Tablets labeled to contain 500 mg: Dilute 2 mL of *Sample stock solution* with water to 100 mL.

For Tablets labeled to contain 1000 mg: Dilute 1 mL of *Sample stock solution* with water to 100 mL.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 232 nm

Blank

For Tablets labeled to contain 500 mg: Dilute 2 mL of *Medium* with water to 100 mL.

For Tablets labeled to contain 1000 mg: Dilute 1 mL of *Medium* with water to 100 mL.

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the concentration (C_i), in mg/mL, of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

D = dilution factor of the *Sample solution*

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = [(C_3 \times V) + [(C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

$$\text{Result}_4 = [(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

C_i = concentration of metformin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)

V = volume of *Medium*, 1000 mL

L = label claim (mg/Tablet)

V_3 = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 15*.

Table 15

Time point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 20
2	4	45–65
3	6	65–85
4	14	NLT 85

The percentages of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Analysis: From the chromatogram of the *Sample solution* obtained in the *Assay*, calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity

r_T = sum of all the peak responses

Acceptance criteria

Individual impurities: NMT 0.1%

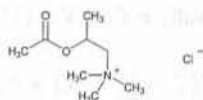
Total impurities: NMT 0.6%

[NOTE—Disregard any peak less than 0.05%, and disregard any peak observed in the blank.]

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one dissolution test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)
 - USP Metformin Hydrochloride RS
 - USP Metformin Related Compound B RS
 - 1-Methylbiguanide hydrochloride.
 - $C_3H_9N_5HCl$ 151.60
 - USP Metformin Related Compound C RS
 - N,N*-Dimethyl-[1,3,5]triazine-2,4,6-triamine.
 - $C_5H_{10}N_6$ 154.17

Methacholine Chloride



$C_8H_{18}ClNO_2$ 195.69
1-Propanaminium, 2-(acetyloxy)-N,N,N-trimethyl-, chloride, (±)-;
(±)-(2-Hydroxypropyl)trimethylammonium chloride acetate [62-51-1].

DEFINITION

Methacholine Chloride contains NLT 98.0% and NMT 101.0% of methacholine chloride ($C_8H_{18}ClNO_2$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191)
Sample solution: 20 mg/mL
Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Mobile phase: 0.5 g/L of methanesulfonic acid in water
Standard solution: 50 µg/mL of USP Methacholine Chloride RS in water

System suitability solution: 10 µg/mL of USP Acetylcholine Chloride RS in *Standard solution*

Sample solution: 50 µg/mL of Methacholine Chloride in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: IC

Detector: Conductivity

Columns

Guard: 4.0-mm x 50-mm; L77¹ packing

Analytical: 4.0-mm x 25-cm; L77¹ packing

Suppressor: Ion-exchange membrane autosuppressor² or a suitable chemical suppression system

Suppressant: Autosuppression

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 2 between acetylcholine and methacholine, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methacholine chloride ($C_8H_{18}ClNO_2$) in the portion of Methacholine Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methacholine Chloride RS in the *Standard solution* (µg/mL)

C_U = concentration of Methacholine Chloride in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-

Jan-2018)

ORGANIC IMPURITIES

Mobile phase: 0.5 g/L of methanesulfonic acid in water
System suitability solution: 1 mg/mL of USP

Methacholine Chloride RS and 1 µg/mL of USP Acetylcholine Chloride RS in water

Standard solution: 1 µg/mL of USP Methacholine Chloride RS in water

Sample solution: 1 mg/mL of Methacholine Chloride in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: IC

Detector: Conductivity

Columns

Guard: 4.0-mm x 50-mm; L77¹ packing

Analytical: 4.0-mm x 25-cm; L77¹ packing

Suppressor: Ion-exchange membrane autosuppressor¹ or a suitable chemical suppression system

Suppressant: Autosuppression

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: Two times the retention time of methacholine

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 2 between acetylcholine and methacholine

Signal-to-noise ratio: NLT 2 for acetylcholine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of beta-methylcholine or acetylcholine in the portion of Methacholine Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of beta-methylcholine or acetylcholine from the *Sample solution*

r_S = peak response of methacholine chloride from the *Standard solution*

C_S = concentration of USP Methacholine Chloride RS in the *Standard solution* (µg/mL)

C_U = concentration of Methacholine Chloride in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

¹ Available as IonPac CS17.

² Available as Cation Self-Regenerating Suppressor (CSRS) from Dionex Inc., or equivalent.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Beta-methylcholine ^a	0.6	0.10
Acetylcholine	0.8	0.10
Methacholine	1.0	—

^a 2-Hydroxy-N,N,N-trimethylpropan-1-aminium chloride.

SPECIFIC TESTS• **LOSS ON DRYING** (731)

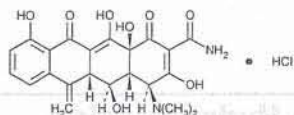
Analysis: Dry a sample at 105° for 4 h.

Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in tight containers.• **USP REFERENCE STANDARDS** (11)

USP Acetylcholine Chloride RS

USP Methacholine Chloride RS

Methacycline Hydrochloride

$C_{22}H_{22}N_2O_8 \cdot HCl$ 478.88

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-, monohydrochloride, [4S-(4 α , 4a α , 5 α , 5a α , 12a α)]-

4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [3963-95-9].

» Methacycline Hydrochloride has a potency equivalent to not less than 832 μ g and not more than 970 μ g of methacycline ($C_{22}H_{22}N_2O_8$) per mg.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Doxycycline Hyclate RS

USP Methacycline Hydrochloride RS

Identification, Ultraviolet Absorption (197U)—

Solution: 20 μ g per mL.

Medium: hydrochloric acid in methanol (1 in 1200).

Absorptivity at 345 nm, calculated on the dried basis, is between 88.4% and 96.4% of the USP Methacycline Hydrochloride RS, the potency of the Reference Standard being taken into account.

Crystallinity (695): meets the requirements.

pH (791): between 2.0 and 3.0, in a solution containing 10 mg of methacycline per mL.

Water Determination, Method I (921): not more than 2.0%.

Assay—

Mobile phase—Prepare a mixture of 0.2 M ammonium oxalate, dimethylformamide, and 0.1 M edetate disodium (11:5:4), adjust with tetrabutylammonium hydroxide, 40 percent in water, to a pH of 7.0, and filter. Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability preparation—Prepare a solution of USP Methacycline Hydrochloride RS and USP Doxycycline Hyclate RS in *Mobile phase* containing about 0.5 mg of each per mL.

Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Methacycline Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL.

Assay preparation—Transfer about 50 mg of Methacycline Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 354-nm detector and a 4.6-mm \times 15-cm column that contains 3.5- μ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.75 for methacycline and 1.0 for doxycycline; and the resolution, *R*, between methacycline and doxycycline is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in μ g, of methacycline ($C_{22}H_{22}N_2O_8$) in each mg of Methacycline Hydrochloride taken by the formula:

$$100(CE/W)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Methacycline Hydrochloride RS in the *Standard preparation*; *E* is the methacycline content, in μ g per mg, of USP Methacycline Hydrochloride RS; *W* is the quantity, in mg, of Methacycline Hydrochloride taken to prepare the *Assay preparation*; and *r_U* and *r_S* are the methacycline peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methacycline Hydrochloride Capsules

» Methacycline Hydrochloride Capsules contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of methacycline ($C_{22}H_{22}N_2O_8$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Doxycycline Hyclate RS

USP Methacycline Hydrochloride RS

Identification—Shake a suitable quantity of Capsule contents with methanol to obtain a solution containing the equivalent of about 1 mg of methacycline per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 60 minutes.

Procedure—Determine the amount of $C_{22}H_{22}N_2O_8 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 345 nm of filtered portions of the solution under test, suitably diluted with water, in comparison with a Standard solution having a known concentra-

tion of USP Methacycline Hydrochloride RS in the same *Medium*.

Tolerances—Not less than 70% (Q) of the labeled amount of $C_{22}H_{22}N_2O_8 \cdot HCl$ is dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements.

Water Determination, Method I (921): not more than 7.5%.

Assay—

Mobile phase, System suitability preparation, and Chromatographic system—Proceed as directed in the Assay under Methacycline Hydrochloride.

Standard preparation—Transfer about 28 mg of USP Methacycline Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of water, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Place no fewer than 5 Capsules in a high-speed, glass blender jar containing an accurately measured volume of water, and blend for 3 to 5 minutes to obtain a stock solution having a concentration of about 2.5 mg of methacycline ($C_{22}H_{22}N_2O_8$) per mL. Filter, transfer 10.0 mL of the filtrate to a 50-mL volumetric flask, add 10 mL of water, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed in the Assay under Methacycline Hydrochloride. Calculate the quantity, in mg, of methacycline ($C_{22}H_{22}N_2O_8$) in each Capsule taken by the formula:

$$5(CE / 1000)(V / N)(r_U / r_S)$$

in which *V* is the volume, in mL, of water used to prepare the stock solution for the Assay preparation; *N* is the number of Capsules taken to prepare the stock solution for the Assay preparation; and the other terms are as defined therein.

Methacycline Hydrochloride Oral Suspension

» Methacycline Hydrochloride Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of methacycline ($C_{22}H_{22}N_2O_8$). It contains one or more suitable and harmless buffers, colors, diluents, dispersants, flavors, and preservatives.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Doxycycline Hyclate RS

USP Methacycline Hydrochloride RS

Identification—To an accurately measured volume of Oral Suspension, equivalent to about 50 mg of methacycline, add 50 mL of methanol, shake, and allow the mixture to settle. Using the clear supernatant as the *Test Solution*, proceed as directed for *Method II* under Identification—Tetracyclines (193).

Uniformity of dosage units (905)—

FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698): meets the requirements.

pH (791): between 6.5 and 8.0.

Assay—

Mobile phase, System suitability preparation, and Chromatographic system—Proceed as directed in the Assay under Methacycline Hydrochloride.

Standard preparation—Transfer about 28 mg of USP Methacycline Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of water, dilute with *Mobile phase* to volume, and mix.

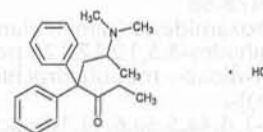
Assay preparation—Transfer an accurately measured quantity of Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 50 mg of methacycline ($C_{22}H_{22}N_2O_8$), to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and filter.

Procedure—Proceed as directed in the Assay under Methacycline Hydrochloride. Calculate the quantity, in mg, of methacycline ($C_{22}H_{22}N_2O_8$) in each mL of the Oral Suspension taken by the formula:

$$100(CE / 1000V)(r_U / r_S)$$

in which *V* is the volume, in mL, of Oral Suspension taken to prepare the Assay preparation; and the other terms are as defined therein.

Methadone Hydrochloride



$C_{21}H_{27}NO \cdot HCl$

345.91

3-Heptanone, 6-(dimethylamino)-4,4-diphenyl-, hydrochloride;

6-(Dimethylamino)-4,4-diphenyl-3-heptanone hydrochloride [1095-90-5].

DEFINITION

Methadone Hydrochloride contains NLT 98.5% and NMT 100.5% of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

• **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191): A solution meets the requirements of the tests.

ASSAY

• PROCEDURE

Sample: 500 mg

Mode: Direct titration

Titrimetric system

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in a mixture of 10 mL of glacial acetic acid and 10 mL of mercuric acetate TS, warming slightly if necessary to dissolve. Cool the solution to room temperature, add 10 mL of dioxane, then add crystal violet TS, and titrate rapidly with *Titrant*. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of *Titrant* is equivalent to 34.59 mg of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$).

Acceptance criteria: 98.5%–100.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **ORDINARY IMPURITIES** (466)
 - Standard solution: Alcohol
 - Sample solution: Alcohol
 - Eluant: Methanol and ammonium hydroxide (100:1.5)
 - Visualization: 3
 - Acceptance criteria: The sum of the intensities of all secondary spots from the *Sample solution* corresponds to NMT 1.0%.

SPECIFIC TESTS

- **PH** (791)
 - Sample solution: 10 mg/mL
 - Acceptance criteria: 4.5–6.5
- **LOSS ON DRYING** (731)
 - Sample: 500 mg
 - Analysis: Dry the *Sample* at 105° for 1 h.
 - Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** (11)
 - USP Methadone Hydrochloride RS

Methadone Hydrochloride Oral Concentrate

DEFINITION

Methadone Hydrochloride Oral Concentrate contains, in each mL, NLT 9.0 mg and NMT 11.0 mg of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$). It contains a suitable preservative and may contain suitable coloring, flavoring, and surface-active agents.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)
 - Sample solution: A volume of Oral Concentrate equivalent to 5 mg of methadone hydrochloride
 - Chromatographic system
 - Developing solvent system: Alcohol, glacial acetic acid, and water (5:3:2)
 - Analysis: Shake the *Sample solution* with 5 mL of sodium carbonate TS, and extract with 5 mL of chloroform. Proceed as directed, using iodoplatinate TS to visualize the spots.
 - Acceptance criteria: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements

ASSAY

- **PROCEDURE**
 - Mobile phase: Acetonitrile and 0.033 M monobasic potassium phosphate (40:60). Adjust with phosphoric acid to a pH of 4.0, filter, and degas.
 - Standard solution: 0.4 mg/mL of USP Methadone Hydrochloride RS in *Mobile phase*
 - Sample stock solution: Nominally 1 mg/mL of methadone hydrochloride from Oral Concentrate in *Mobile phase*
 - Sample solution: 0.4 mg/mL of methadone hydrochloride in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)
 Mode: LC
 Detector: UV 254 nm
 Column: 3.9-mm × 30-cm; packing L11
 Flow rate: 2 mL/min
 Injection volume: 10 μ L
 System suitability
 Sample: *Standard solution*
 Suitability requirements
 Column efficiency: NLT 1500 theoretical plates
 Tailing factor: NMT 2.0
 Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*
 Calculate the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) in the portion of Oral Concentrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times L$$

r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Methadone Hydrochloride RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of the *Sample solution* (mg/mL)
 L = label claim (mg/mL)
 Acceptance criteria: 9.0–11.0 mg/mL

SPECIFIC TESTS

- **PH** (791): 1.0–6.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and store at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be diluted with water or other liquid to 30 mL or more before administration.
- **USP REFERENCE STANDARDS** (11)
 - USP Methadone Hydrochloride RS

Methadone Hydrochloride Injection

DEFINITION

Methadone Hydrochloride Injection is a sterile solution of Methadone Hydrochloride in Water for Injection. It contains, in each mL, NLT 9.5 mg and NMT 10.5 mg of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$).

IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** (181): Meets the requirements

ASSAY

- **PROCEDURE**
 - Internal standard solution: 5 mg/mL of procaine in methylene chloride
 - Standard solution: Transfer 10 mg of USP Methadone Hydrochloride RS to a 60-mL separator. Add 1 mL of water and 2 mL of 0.5 N sodium hydroxide, and extract with three 10-mL portions of methylene chloride, combining the extracts in a vessel containing about 3 g of anhydrous sodium sulfate. Transfer 2.0 mL of *Internal standard solution* to the vessel containing the extracts, insert the stopper, and mix. Decant 15 mL of the methylene chloride solution to a test tube, and evaporate to a volume of 2–3 mL using vacuum or a stream of nitrogen.

Sample solution: Transfer 1.0 mL of Injection, equivalent to 10 mg of methadone hydrochloride, to a 60-mL separator. Add 2 mL of 0.5 N sodium hydroxide, and extract with three 10-mL portions of methylene chloride, combining the extracts in a vessel containing about 3 g of anhydrous sodium sulfate. Transfer 2.0 mL of *Internal standard solution* to the vessel containing the extracts, insert the stopper, and mix. Decant 15 mL of the methylene chloride solution to a test tube, and evaporate to a volume of 2–3 mL using vacuum or a stream of nitrogen.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: Glass column, 1.2-m long and 4-mm in diameter; packed with 3% phase G2 on 100- to 200-mesh support S1A

Temperatures

Column: 170°

Injection port: 225°

Detector: 240°

Carrier gas: Dry helium

Flow rate: 55 mL/min

Injection volume: Containing 5 µg of methadone

System suitability

Sample: *Standard solution* (six replicate injections)

Suitability requirements

Resolution: NLT 5.0 between methadone and procaine

Coefficient of variation: NMT 1% in the ratios of the peak areas of methadone to the peak area of procaine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in mg, of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) in each mL of Injection taken:

$$\text{Result} = (R_U/R_S) \times W \times 100$$

R_U = peak area ratio of methadone to procaine from the *Sample solution*

R_S = peak area ratio of methadone to procaine from the *Standard solution*

W = weight, in mg, of USP Methadone Hydrochloride RS in the *Standard solution*

Acceptance criteria: 9.5–10.5 mg/mL

SPECIFIC TESTS

• **pH (791):** 3.0–6.5

• **BACTERIAL ENDOTOXINS TEST (85):** NMT 8.8 USP Endotoxin Units/mg of methadone hydrochloride

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose, light-resistant containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS (11)**

USP Endotoxin RS

USP Methadone Hydrochloride RS

Methadone Hydrochloride Oral Solution

DEFINITION

Methadone Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$).

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

Sample solution: A volume of Oral Solution equivalent to 5 mg of methadone hydrochloride

Developing solvent system: Alcohol, glacial acetic acid, and water (5:3:2)

Analysis: Shake the *Sample solution* with 5 mL of sodium carbonate TS, and extract with 5 mL of chloroform. Proceed as directed using iodoplatinate TS to visualize the spots.

Acceptance criteria: Meets the requirements

• B. IDENTIFICATION TESTS—GENERAL, Chloride (191):

Meets the requirements

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile and 0.033 M monobasic potassium phosphate (40:60). Adjust dropwise with phosphoric acid to a pH of 4.0.

Internal standard solution: 250 µg/mL of pyrilamine maleate

Standard solution: Transfer 20 mg of USP Methadone Hydrochloride RS to a 25-mL volumetric flask. Add 2.0 mL of *Internal standard solution*, and dilute with water to volume.

Sample solution: Transfer a volume of Oral Solution equivalent to 20 mg of methadone hydrochloride to a 125-mL separator. Extract the specimen with two 50-mL portions of ether, collecting the ether extracts in a second separator. Wash the combined ether extracts with 2 mL of water, and discard the ether extract. Transfer the aqueous wash and the aqueous specimen to a 25-mL volumetric flask. Add 2.0 mL of *Internal standard solution*, and dilute with water to volume. Pass the solution through a filter of 5-µm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L11

Flow rate: 1.3 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

The retention times for the internal standard and methadone hydrochloride are about 5.5 and 9 min, respectively.

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) in the Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of methadone hydrochloride to the internal standard from the *Sample solution*

R_S = peak response ratio of methadone hydrochloride to the internal standard from the *Standard solution*

C_S = concentration of USP Methadone Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methadone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **ALCOHOL DETERMINATION, Method II (611)** (if present): 90.0%–115.0% of the labeled amount of C_2H_5OH , deter-

mined by the gas chromatographic procedure using acetone as the internal standard

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements for Oral Solution packaged in single-unit containers
- **DELIVERABLE VOLUME (698):** Meets the requirements for Oral Solution packaged in multiple-unit containers

SPECIFIC TESTS

- **PH (791):** 1.0–4.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Methadone Hydrochloride RS

Methadone Hydrochloride Tablets

DEFINITION

Methadone Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**
Sample: Equivalent to 5 mg of methadone hydrochloride from a quantity of finely powdered Tablets
Chromatographic system
Developing solvent system: Alcohol, glacial acetic acid, and water (5:3:2)
Analysis: Shake the *Sample* with 5 mL of sodium carbonate TS, and extract with 5 mL of chloroform. Proceed as directed using iodoplatinate TS to visualize the spots.
Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**
Mobile phase: Acetonitrile and 0.03 M monobasic potassium phosphate (40:60). Adjust with phosphoric acid to a pH of 3.2.
Standard solution: 0.4 mg/mL of USP Methadone Hydrochloride RS in *Mobile phase*
Sample solution: 0.4 mg/mL of methadone hydrochloride in *Mobile phase*. Prepare by transferring an amount of finely powdered Tablets (NLT 20) equivalent to 10 mg of methadone hydrochloride to a 25-mL volumetric flask, add 10 mL of *Mobile phase*, and sonicate briefly. Shake by mechanical means for 15 min, dilute with *Mobile phase* to volume, and filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

- Mode:** LC
- Detector:** UV 254 nm
- Column:** 3.9-mm \times 30-cm; packing L11
- Flow rate:** 1.5 mL/min
- Injection volume:** 10 μ L
- System suitability**
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
- r_S = peak response from the *Standard solution*
- C_S = concentration of USP Methadone Hydrochloride RS in the *Standard solution* (mg/mL)
- C_U = nominal concentration of methadone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

- **DISSOLUTION (711)**
Medium: Water; 500 mL
Apparatus 1: 100 rpm
Time: 45 min
Standard solution: USP Methadone Hydrochloride RS in *Medium*
Sample solution: *Sample* per *Dissolution* (711).
Instrumental conditions
Mode: Vis
Analytical wavelength: Maximum at about 405 nm
Analysis: Filter a portion of the *Sample solution*, and pipet a volume of the filtrate equivalent to about 400 μ g of methadone hydrochloride into a suitable separator. Add 1 mL of glacial acetic acid and 20 mL of a solution of bromocresol purple prepared by dissolving 200 mg of bromocresol purple in 1000 mL of dilute glacial acetic acid (1 in 50). Mix, and extract with 20.0 mL of chloroform.
Determine the amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) dissolved from visible absorbances at the wavelength of maximum absorbance of the chloroform extract so obtained in comparison with the chloroform extract similarly prepared from the *Standard solution*.
Tolerances: NLT 75% (Q) of the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS** (11)
USP Methadone Hydrochloride RS

Methadone Hydrochloride Tablets for Oral Suspension

DEFINITION

Methadone Hydrochloride Tablets for Oral Suspension contain NLT 93.0% and NMT 107.0% of the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

Sample solution: Shake a quantity of powdered Tablets for Oral Suspension equivalent to 5 mg of methadone hydrochloride with 5 mL of sodium carbonate TS, and extract with 5 mL of chloroform.

Developing solvent system: Alcohol, glacial acetic acid, and water (5:3:2)

Analysis: Proceed as directed, using iodoplatinate TS to visualize the spots.

Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and 0.03 M monobasic potassium phosphate (40:60). Adjust with phosphoric acid to a pH of 3.2.

Standard solution: 0.4 mg/mL of USP Methadone Hydrochloride RS in *Mobile phase*

Sample solution: 0.4 mg/mL of methadone hydrochloride in *Mobile phase*. Prepare by transferring an amount of finely powdered Tablets for Oral Suspension (NLT 20) equivalent to 10 mg of methadone hydrochloride to a 25-mL volumetric flask, adding 10 mL of *Mobile phase*, and sonicating briefly. Shake by mechanical means for 15 min, dilute with *Mobile phase* to volume, and filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; packing L11

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$) in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- r_u = peak response from the *Sample solution*
 r_s = peak response from the *Standard solution*
 C_s = concentration of USP Methadone Hydrochloride RS in the *Standard solution* (mg/mL)
 C_u = nominal concentration of methadone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

- **DISINTEGRATION** (701)

Time: 15 min

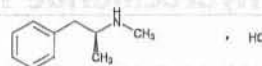
Acceptance criteria: Meet the requirements

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label the Tablets for Oral Suspension to indicate that they are intended for dispersion in a liquid before oral administration of the prescribed dose.
- **USP REFERENCE STANDARDS** (11)
USP Methadone Hydrochloride RS

Methamphetamine Hydrochloride



$C_{10}H_{15}N \cdot HCl$ 185.69
 Benzeneethanamine, *N*, α -dimethyl-, hydrochloride, (S)-;
 (+)-(S)-*N*, α -Dimethylphenethylamine hydrochloride
 [51-57-0].

DEFINITION

Methamphetamine Hydrochloride contains NLT 98.5% and NMT 100.5% of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191)

ASSAY

- **PROCEDURE**

Mobile phase: Prepare a degassed solution of 1.1 g of sodium 1-heptanesulfonate in a mixture of water, methanol, and diluted glacial acetic acid (7 in 50) (575:400:25). Adjust with acetic acid to a pH of 3.3 \pm 0.1, if necessary. Filter through a 0.5- μ m disk.

Standard solution: 0.2 mg/mL of USP Methamphetamine Hydrochloride RS in 0.12 M phosphoric acid. Sonicate if necessary.

Sample solution: 0.2 mg/mL of Methamphetamine Hydrochloride in 0.12 M phosphoric acid

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 257 nm

Column: 3.9-mm \times 30.0-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$) in the portion of Methamphetamine Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- r_u = peak response from the *Sample solution*

- r_s = peak response from the *Standard solution*
 C_s = concentration of USP Methamphetamine Hydrochloride RS in the *Standard solution* (mg/mL)
 C_u = concentration of Methamphetamine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–100.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **ORDINARY IMPURITIES** (466)
Standard solution and *Sample solution*: Chloroform
Eluent: Chloroform, cyclohexane, and diethylamine (5:4:1)
Visualization: 1
 Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** (741): 171°–175°
- **OPTICAL ROTATION, Specific Rotation** (781S)
Sample solution: 20 mg/mL in water
 Acceptance criteria: Between +16° and +19°
- **LOSS ON DRYING** (731)
Analysis: Dry at 105° for 2 h.
 Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
 USP Methamphetamine Hydrochloride RS

Methamphetamine Hydrochloride Tablets

DEFINITION

Methamphetamine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$).

IDENTIFICATION

- **A.** The UV absorption spectrum of the *Sample solution*, prepared as described in *Procedure for content uniformity* in *Uniformity of Dosage Units*, exhibits maxima and minima at the same wavelengths as those of the *Standard solution*, concomitantly measured.

ASSAY

• PROCEDURE

Mobile phase: Prepare a degassed solution of 1.1 g of sodium 1-heptanesulfonate in a mixture of water, methanol, and diluted glacial acetic acid (7 in 50) (575:400:25). Adjust with acetic acid to a pH of 3.3 ± 0.1 , if necessary. Filter through a 0.5- μ m disk.

Standard solution: 0.2 mg/mL of USP Methamphetamine Hydrochloride RS in 0.12 M phosphoric acid. Sonicate if necessary.

Sample solution: Transfer a portion of fine powder from NLT 20 Tablets, nominally equivalent to about 10 mg of methamphetamine hydrochloride, to a 50-mL volumetric flask. Add 20 mL of 0.12 M phosphoric acid, and sonicate for 5 min. Dilute with 0.12 M phosphoric acid to volume, and filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 257 nm

Column: 3.9-mm \times 30.0-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Methamphetamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of methamphetamine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Mobile phase: Acetonitrile and dilute perchloric acid (1 in 20) (300:700). Filter, and degas.

Sample solution: Filter aliquots of the solution in the test, and dilute 2:1 with 0.15 M perchloric acid.

Standard solution: Dissolve USP Methamphetamine Hydrochloride RS in water to obtain a concentration similar to the one expected in the *Sample solution*. Dilute 2:1 with 0.15 M perchloric acid.

Chromatographic system

Mode: LC

Detector: UV 211 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 2.5 mL/min

Injection volume: 100 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of the labeled amount of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$) dissolved by comparing the major peak response of the *Sample solution* with that of the *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Solution A: Shake 250 mL of 0.1 N sulfuric acid with 25 mL of chloroform for 10 min. Allow to stand for 1 h with occasional shaking. Drain off the chloroform, and retain the chloroform-saturated sulfuric acid in a stoppered flask.

Standard solution: 0.5 mg/mL of USP

Methamphetamine Hydrochloride RS in *Solution A*

Sample solution: Place 1 Tablet in a 125-mL separator. Add 15 mL of water, and shake by mechani-

cal means for 15 min to dissolve. Add 2.5 mL of 1 N sodium hydroxide, and shake. Extract the liberated methamphetamine with four 10-mL portions of chloroform, collecting the chloroform extracts in a second 125-mL separator. Transfer 10.0 mL of *Solution A* to the second separator, and shake by mechanical means for 10 min. Allow the layers to separate, and collect the aqueous layer.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 257 nm

Cell: 1 cm

Blank: *Solution A*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of the labeled amount of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Methamphetamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methamphetamine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

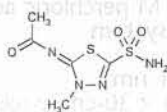
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP Methamphetamine Hydrochloride RS

Methazolamide



$C_5H_8N_4O_3S_2$ 236.27

Acetamide, N-[5-(aminosulfonyl)-3-methyl-1,3,4-thiadiazol-2(3H)-ylidene]-.

N-(4-methyl-2-sulfamoyl- Δ^2 -1,3,4-thiadiazolin-5-ylidene)-acetamide [554-57-4].

» Methazolamide contains not less than 98.0 percent and not more than 102.0 percent of $C_5H_8N_4O_3S_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards (11)—

USP Methazolamide RS

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 10 μ g per mL.

Medium: sodium hydroxide solution in water (1 in 250).

Loss on drying (731)—Dry it at 105° for 2 hours; it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.1%.

Selenium (291): 0.003%, a 200-mg specimen being used.

Delete the following:

• **Heavy metals, Method II (231):** 0.002%. (Official 1-Jan-2018)

Assay—

Buffer solution—Dissolve 1.80 g of anhydrous sodium acetate in 1 L of water. Adjust, if necessary, with glacial acetic acid to a pH of 4.5 ± 0.2 .

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (86:14). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve about 20 mg of USP Methazolamide RS, accurately weighed, in 20 mL of acetonitrile contained in a 200-mL volumetric flask. Dilute with *Buffer solution* to volume, and mix. Quantitatively dilute an accurately measured volume of this solution with *Buffer solution* to obtain a solution having a known concentration of about 50 μ g of USP Methazolamide RS per mL.

Resolution solution—Prepare a solution of acetaminophen and USP Methazolamide RS in acetonitrile containing 0.3 mg of acetaminophen and 0.5 mg of methazolamide per mL. Quantitatively dilute an accurately measured volume of this solution with *Buffer solution* to obtain a solution containing 30 μ g of acetaminophen and 50 μ g of methazolamide per mL.

Assay preparation—Transfer about 100 mg of Methazolamide, accurately weighed, to a 200-mL volumetric flask, dissolve in 20 mL of acetonitrile, dilute with *Buffer solution* to volume, and mix. Quantitatively dilute an accurately measured volume of this solution with *Buffer solution* to obtain a solution having a known concentration of about 50 μ g per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 265-nm detector and a 3.9-mm \times 15.0-cm column containing packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for acetaminophen and 1.0 for methazolamide, the resolution, R , between the acetaminophen peak and the methazolamide peak is not less than 4.0, and the tailing factor is not more than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the responses for the major peaks. Calculate the quantity, in mg, of $C_5H_8N_4O_3S_2$ in the portion of Methazolamide taken by the formula:

$$2C(r_U/r_S)$$

in which C is the concentration, in μ g per mL, of USP Methazolamide RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methazolamide Tablets

» Methazolamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of

the labeled amount of methazolamide ($C_5H_8N_4O_3S_2$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Methazolamide RS

Identification—

A: Extract a quantity of finely powdered Tablets, equivalent to about 250 mg of methazolamide, with about 50 mL of acetone. Filter, and add solvent hexane until a heavy white precipitate is formed. Collect the solid on a filter, and dry: the IR absorption spectrum of a potassium bromide dispersion of the methazolamide so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Methazolamide RS.

B: Dissolve about 100 mg of the dried solid obtained in Identification test A in 5 mL of 1 N sodium hydroxide, and add 5 mL of a mixture of 1 g of hydroxylamine hydrochloride and 500 mg of cupric sulfate in 100 mL of water. Heat the solution on a steam bath for 15 minutes: the solution turns dark amber, then a black precipitate is formed.

C: The retention time of the major peak in the chromatogram of the Assay preparation corresponds to the chromatogram of the Standard preparation, as obtained in the Assay.

Dissolution (711)—

Medium: pH 4.5 acetate buffer, prepared by mixing 2.99 g of sodium acetate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of a solution having a pH of 4.5; 900 mL.

Apparatus 2: 75 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_5H_8N_4O_3S_2$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 252 nm of filtered portions of the solution under test, suitably diluted with pH 4.5 acetate buffer, in comparison with a Standard solution having a known concentration of USP Methazolamide RS in the same Medium.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_5H_8N_4O_3S_2$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

pH 2.5 Buffer—Transfer 16.8 mL of dibutylamine to a beaker containing 70 mL of water. Adjust with phosphoric acid to a pH of 2.5, dilute with water to 100 mL, and mix.

Mobile phase—Prepare a mixture of water, methanol, and pH 2.5 Buffer (375:15:6). Make adjustments if necessary (see System Suitability under Chromatography (621)).

pH 4.5 Acetate buffer—Dissolve 2.99 g of sodium acetate and 1.66 mL of glacial acetic acid in water, dilute with water to 1000 mL, and mix. Adjust, if necessary, with glacial acetic acid or sodium hydroxide to a pH of 4.5.

Standard preparation—Dissolve an accurately weighed quantity of USP Methazolamide RS in methanol to obtain a solution having a concentration of about 0.5 mg per mL. Dilute this solution quantitatively, and stepwise if necessary, with pH 4.5 Acetate buffer to obtain a solution having a known concentration of about 50 µg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of methazolamide, to a 250-mL volumetric flask, add 65 mL of pH 4.5 Acetate buffer, and sonicate to dissolve. Add 65 mL of methanol, and sonicate again until dissolved. Dilute with pH 4.5 Acetate

buffer to volume, mix, and filter. Dilute an accurately measured volume of the filtrate with pH 4.5 Acetate buffer to obtain a solution having a concentration of about 50 µg of methazolamide per mL.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 252-nm detector and an 8-mm × 10-cm column that contains packing L10. The flow rate is about 2 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of methazolamide ($C_5H_8N_4O_3S_2$) in the portion of Tablets taken by the formula:

$$C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Methazolamide RS in the Standard preparation; and r_U and r_S are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

Methdilazine Hydrochloride

$C_{18}H_{20}N_2S \cdot HCl$ 332.89

10H-Phenothiazine, 10-[(1-methyl-3-pyrrolidiny)methyl]-, monohydrochloride.

10-[(1-Methyl-3-pyrrolidiny)methyl]phenothiazine monohydrochloride [1229-35-2].

» Methdilazine Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of $C_{18}H_{20}N_2S \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Methdilazine Hydrochloride RS

NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 5 µg per mL.

Medium: water.

C: A solution of it responds to the tests for Chloride (191).

Melting range, Class I (741): between 184° and 190°.

pH (791): between 4.8 and 6.0, in a solution (1 in 100).

Loss on drying (731)—Dry it in vacuum at 65° for 16 hours: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.5%.

Selenium (291)—The absorbance of the solution from the Test Solution, prepared with 100 mg of Methdilazine Hydrochloride and 100 mg of magnesium oxide, is not greater than one-half that from the Standard Solution (0.003%).

Delete the following:

• **Heavy metals, Method II (231):** 0.002%. (Official 1-Jan-2016)

Ordinary impurities (466)—

Test solution: methanol.

Standard solution: methanol.

Application volume: 10 μ L.

Eluant: a mixture of toluene, isopropyl alcohol, and ammonium hydroxide (70:29:1), in a nonequilibrated chamber.

Visualization: 5.

Assay—Transfer about 100 mg of Methdilazine Hydrochloride, accurately weighed, to a 1000-mL volumetric flask, add water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Methdilazine Hydrochloride RS in the same medium having a known concentration of about 5 μ g per mL, in 1-cm cells at 252 and 275 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{18}H_{20}N_2S \cdot HCl$ in the portion of Methdilazine Hydrochloride taken by the formula:

$$20C(A_{252} - A_{275})_U / (A_{252} - A_{275})_S$$

in which C is the concentration, in μ g per mL, of USP Methdilazine Hydrochloride RS in the Standard solution; and the parenthetical expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution of Methdilazine Hydrochloride (U) and the Standard solution (S), respectively.

Methdilazine Hydrochloride Oral Solution

» Methdilazine Hydrochloride Oral Solution contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of methdilazine hydrochloride ($C_{18}H_{20}N_2S \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Methdilazine Hydrochloride RS

NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

Identification—Transfer a volume of Oral Solution, equivalent to about 4 mg of methdilazine hydrochloride, to a 60-mL separator, add 5 mL of 0.1 N hydrochloric acid, and extract with 10 mL of ether, discarding the extract. Add 10 mL of sodium bicarbonate solution (1 in 10) to the separator, and extract with 3 mL of chloroform. Filter the extract through a pledget of cotton. Evaporate the chloroform, carefully removing the last trace of solvent in a small vacuum flask: the IR absorption spectrum of a potassium bromide dispersion of the methdilazine so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Methdilazine Hydrochloride RS that has been treated in the same manner.

pH (791): between 3.3 and 4.1.

Alcohol Determination, Method II (611): between 6.5% and 7.5% of C_2H_5OH .

Assay—

Standard preparation—Dissolve a suitable quantity of USP Methdilazine Hydrochloride, accurately weighed, in chloroform, and quantitatively dilute with chloroform to obtain a solution having a known concentration of about 400 μ g per mL.

Assay preparation—Transfer a volume of Oral Solution, equivalent to about 4 mg of methdilazine hydrochloride, to a 60-mL separator, add 10 mL of a saturated solution of sodium chloride, and extract with three 10-mL portions of chloroform, transferring the extracts to a 100-mL volumetric flask.

Procedure—Transfer 10.0 mL of Standard preparation to a 100-mL volumetric flask, and add 20 mL of chloroform. To this flask and to the flask containing the Assay preparation add 4.0 mL of buffered palladium chloride TS, dilute with alcohol to volume, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 460 nm, with a suitable spectrophotometer, using a mixture of 30 mL of chloroform, 4 mL of palladium chloride TS, and 66 mL of alcohol as the blank. Calculate the quantity, in mg, of methdilazine hydrochloride ($C_{18}H_{20}N_2S \cdot HCl$) in each mL of the Oral Solution taken by the formula:

$$(0.01C / V)(A_U / A_S)$$

in which C is the concentration, in μ g per mL, of USP Methdilazine Hydrochloride RS in the Standard preparation; V is the volume, in mL, of Oral Solution taken; and A_U and A_S are the absorbances of the solutions from the Assay preparation and the Standard preparation, respectively.

Methdilazine Hydrochloride Tablets

» Methdilazine Hydrochloride Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of methdilazine hydrochloride ($C_{18}H_{20}N_2S \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Methdilazine Hydrochloride RS

NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

Identification—Transfer a portion of finely powdered Tablets, equivalent to about 8 mg of methdilazine hydrochloride, to a 60-mL separator, add 10 mL of sodium bicarbonate solution (1 in 10), and extract with 3 mL of chloroform. Filter the extract through a pledget of cotton. Evaporate the chloroform, carefully removing the last trace of solvent in a small vacuum flask: the IR absorption spectrum of a potassium bromide dispersion of the methdilazine so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Methdilazine Hydrochloride RS, similarly treated and measured.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{18}H_{20}N_2S \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 252 nm of filtered portions of the solution under test, suitably diluted with Dissolution Medium, if necessary, in comparison with a Standard solution having a

known concentration of USP Methdilazine Hydrochloride RS in the same Medium.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{18}H_{20}N_2S \cdot HCl$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Standard preparation—Dissolve a suitable quantity of USP Methdilazine Hydrochloride RS, accurately weighed, in chloroform, and dilute quantitatively with chloroform to obtain a solution having a known concentration of about 400 µg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets, and transfer an accurately weighed portion of the powder, equivalent to about 80 mg of methdilazine hydrochloride, to a 200-mL volumetric flask. Add 60 mL of chloroform, shake for 20 minutes, dilute with chloroform to volume, and mix. Filter, discarding the first 15 mL of the filtrate. Use the subsequent filtrate as directed in the Procedure.

Procedure—Into three separate 100-mL volumetric flasks transfer 10.0 mL each of the *Standard preparation*, the *Assay preparation*, and chloroform to provide the blank. To each flask add 20 mL of chloroform and 4.0 mL of buffered palladium chloride TS, dilute with alcohol to volume, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 460 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of methdilazine hydrochloride ($C_{18}H_{20}N_2S \cdot HCl$) in the portion of Tablets taken by the formula:

$$0.2C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Methdilazine Hydrochloride RS in the *Standard preparation*; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Methenamine



$C_6H_{12}N_4$ 140.19
1,3,5,7-Tetraazatricyclo[3.3.1.1^{3,7}]decane;
Hexamethylenetetramine [100-97-0].

DEFINITION

Methenamine, dried over phosphorus pentoxide for 4 h, contains NLT 99.0% and NMT 100.5% of methenamine ($C_6H_{12}N_4$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

• B.

Analysis: Heat a solution (1 in 10) with 2 N sulfuric acid.

Acceptance criteria: Formaldehyde is liberated, recognizable by its odor and by its darkening of paper moistened with silver ammonium nitrate TS. On the subsequent addition of an excess of 1 N sodium hydroxide to the solution, ammonia is evolved.

ASSAY

• PROCEDURE

Chromotropic acid spot test solution: Suspend 100 mg of chromotropic acid in 2 mL of water, and

cautiously add 3 mL of sulfuric acid. Allow to cool, and add 25 mL of sulfuric acid. If excessive heat generated during mixing causes a violet color to appear in the solution, discard the solution and prepare another, taking precautions to avoid excessive heat.

Sample solution: Transfer 1 g of Methenamine, previously dried, to a beaker. Add 40.0 mL of 1 N sulfuric acid VS, and heat to a gentle boil, adding water from time to time if necessary, until the formaldehyde has been expelled. Test for the absence of formaldehyde by adding a drop of the assay solution to a glass fiber filter disk, on a watch glass, on which has previously been placed 3 or 4 drops of *Chromotropic acid spot test solution*. Formaldehyde produces a violet color with this reagent. Repeat the test until no violet color is obtained on the warmed test filter disk upon comparison with a blank filter disk to which no assay specimen is added. Cool, add 20 mL of water, then add methyl red TS.

Titrimetric system

Mode: Residual titration

Titrant: 1 N sulfuric acid VS

Back-titrant: 1 N sodium hydroxide VS

Endpoint detection: Visual

Analysis: Titrate the excess acid in the *Sample solution* with 1 N sodium hydroxide VS. Perform a blank determination (see *Titrimetry* (541), *Residual Titrations*). Each mL of 1 N sulfuric acid is equivalent to 35.05 mg of methenamine ($C_6H_{12}N_4$).

Acceptance criteria: 99.0%–100.5%

IMPURITIES

• RESIDUE ON IGNITION (281): NMT 0.1%

• CHLORIDE AND SULFATE, Chloride (221)

Sample: 1.0 g

Acceptance criteria: 0.014%; shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid.

• SULFATE

Sample solution: 20 mg/mL

Analysis: 10 mL of the *Sample solution*, acidified with 5 drops of hydrochloric acid. Add 5 drops of barium chloride TS.

Acceptance criteria: No turbidity is produced within 1 min.

Delete the following:

• HEAVY METALS, Method I (231)

Test preparation: 2 g in 10 mL of water

Analysis: Add 2 mL of 3 N hydrochloric acid, and dilute with water to 25 mL. Proceed as directed, except use glacial acetic acid to adjust the pH.

Acceptance criteria: NMT 10 ppm • (Official 1-Jan-2018)

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry over phosphorus pentoxide for 4 h.

Acceptance criteria: NMT 2.0%

• AMMONIUM SALTS

Sample solution: 50 mg/mL

Analysis: Add to 10 mL of the *Sample solution* 1 mL of alkaline mercuric-potassium iodide TS.

Acceptance criteria: The mixture is not darker in color than a mixture of 1 mL of the reagent and 10 mL of water.

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**
USP Methenamine RS

Methenamine Oral Solution

DEFINITION

Methenamine Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of methenamine ($C_6H_{12}N_4$).

IDENTIFICATION

- **A.**
Sample solution: Heat a volume of Oral Solution, equivalent to 1 g of methenamine, with 10 mL of 2 N sulfuric acid.
Acceptance criteria: Formaldehyde is liberated, recognizable by its odor and by its darkening of paper moistened with silver ammonium nitrate TS. On the subsequent addition of an excess of 1 N sodium hydroxide to the solution, ammonia is evolved.

ASSAY

• PROCEDURE

Chromotropic acid solution: Mix 100 mg of chromotropic acid with 50 mL of water in a 100-mL volumetric flask. Cool in an ice bath and, while cooling, cautiously and slowly add 50 mL of sulfuric acid. Allow the solution to reach room temperature, and add dilute sulfuric acid (1 in 2) to volume. If excessive heat generated during mixing causes a violet color to appear in the solution, discard the solution and prepare another, taking precautions to avoid excessive heat.

Standard stock solution: 0.05 mg/mL of USP Methenamine RS

Standard solution: 1 µg/mL of USP Methenamine RS prepared as follows. Transfer 2.0 mL of *Standard stock solution* to a 100-mL volumetric flask, then add 25 mL of *Chromotropic acid solution* and 50 mL of dilute sulfuric acid (1 in 2). Place the flask in a boiling water bath for 30 min, accurately timed, then remove it from the bath. Cool immediately to room temperature, then add dilute sulfuric acid (1 in 2) to volume.

Standard blank: Transfer 2.0 mL of *Standard stock solution* to a 100-mL volumetric flask, then add 75 mL of dilute sulfuric acid (1 in 2). Place the flask in a boiling water bath for 30 min, accurately timed, then remove it from the bath. Cool immediately to room temperature, then add dilute sulfuric acid (1 in 2) to volume.

Sample stock solution: Nominally 60 µg/mL of methenamine from Oral Solution

Sample solution: Nominally 1.2 µg/mL of methenamine prepared as follows. Transfer 2.0-mL of *Sample stock solution* to a 100-mL volumetric flask, then add 25 mL of *Chromotropic acid solution* and 50 mL of dilute sulfuric acid (1 in 2). Place the flask in a boiling water bath for 30 min, accurately timed, then remove it from the bath. Cool immediately to room temperature, then add dilute sulfuric acid (1 in 2) to volume.

Sample blank: Transfer 2.0 mL of *Sample stock solution* to a 100-mL volumetric flask, then 75 mL of dilute sulfuric acid (1 in 2). Place the flask in a boiling water bath for 30 min, accurately timed, then remove it from the bath. Cool immediately to room temperature, and add dilute sulfuric acid (1 in 2) to volume.

Instrumental conditions

Mode: Vis

Analytical wavelength: Maxima at about 570 nm

Cell: 1 cm

Blank: Dilute sulfuric acid (1 in 2)

Analysis

Samples: *Standard solution*, *Standard blank*, *Sample solution*, *Sample blank*, and *Blank*

Calculate the percentage of the labeled amount of methenamine ($C_6H_{12}N_4$) in each mL of Oral Solution taken:

$$\text{Result} = [(A_U - B_U)/(A_S - B_S)] \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

B_U = absorbance of the *Sample blank*

A_S = absorbance of the *Standard solution*

B_S = absorbance of the *Standard blank*

C_S = concentration of USP Methenamine RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of methenamine in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

- **ALCOHOL DETERMINATION, Method I (611):** 90.0%–110.0% of the labeled amount of C_2H_5OH

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements for Oral Solution packaged in single-unit containers
- **DELIVERABLE VOLUME (698):** Meets the requirements for Oral Solution packaged in multiple-unit containers

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Methenamine RS

Methenamine Tablets

DEFINITION

Methenamine Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of methenamine ($C_6H_{12}N_4$).

IDENTIFICATION

- **A.**
Sample: 500 mg of powdered Tablets
Analysis: Dissolve the *Sample* in 10 mL of water, add 10 mL of 2 N sulfuric acid, and heat.
Acceptance criteria: Formaldehyde is liberated, recognizable by its odor and by its darkening of paper moistened with silver ammonium nitrate TS. On the subsequent addition of an excess of 1 N sodium hydroxide to the solution, ammonia is evolved.

ASSAY

• PROCEDURE

Chromotropic acid solution: Mix 100 mg of chromotropic acid with 50 mL of water in a 100-mL volumetric flask. Cool in an ice bath and, while cooling, cautiously and slowly add 50 mL of sulfuric acid. Allow the solution to reach room temperature, and add dilute sulfuric acid (1 in 2) to volume. If excessive heat generated during mixing causes a violet color to appear in the solution, discard the solution and prepare another, taking precautions to avoid excessive heat.

Standard stock solution: 50 µg/mL of USP Methenamine RS

Standard solution: 1 µg/mL of USP Methenamine RS prepared as follows. Transfer 2.0 mL of *Standard stock solution* to a 100-mL volumetric flask, add 25 mL of *Chromotropic acid solution* and 50 mL of dilute sulfuric acid (1 in 2). Place the flask in a boiling water bath for 30 min, accurately timed, then remove it from the bath. Cool immediately to room temperature, then add dilute sulfuric acid (1 in 2) to volume.

Standard blank: Transfer 2.0 mL of *Standard stock solution* to a 100-mL volumetric flask, then add 75 mL of dilute sulfuric acid (1 in 2). Place the flask in a boiling

water bath for 30 min, accurately timed, then remove it from the bath. Cool immediately to room temperature, then add dilute sulfuric acid (1 in 2) to volume.

Sample stock solution: Nominally 50 µg/mL of methenamine prepared as follows. Transfer an equivalent to 500 mg of methenamine from powdered Tablets (NLT 20) to a 250-mL volumetric flask. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Transfer 25.0 mL of the subsequent filtrate to a 1000-mL volumetric flask. Dilute with water to volume.

Sample solution: Nominally 1 µg/mL of methenamine prepared as follows. Transfer 2.0-mL of *Sample stock solution* to a 100-mL volumetric flask, then add 25 mL of *Chromotropic acid solution* and 50 mL of dilute sulfuric acid (1 in 2). Place the flask in a boiling water bath for 30 min, accurately timed, then remove it from the bath. Cool immediately to room temperature, then add dilute sulfuric acid (1 in 2) to volume.

Sample blank: Transfer 2.0 mL of *Sample stock solution* to a 100-mL volumetric flask, then add 75 mL of dilute sulfuric acid (1 in 2). Place the flask in a boiling water bath for 30 min, accurately timed, then remove it from the bath. Cool immediately to room temperature, then add dilute sulfuric acid (1 in 2) to volume.

Instrumental conditions

Mode: Vis

Analytical wavelength: Maxima at about 570 nm

Cell: 1 cm

Blank: Dilute sulfuric acid (1 in 2)

Analysis

Samples: *Standard solution*, *Standard blank*, *Sample solution*, *Sample blank*, and *Blank*

Calculate the percentage of the labeled amount of methenamine ($C_6H_{12}N_4$) in the portion of Tablets taken:

$$\text{Result} = [(A_U - B_U)/(A_S - B_S)] \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

B_U = absorbance of the *Sample blank*

A_S = absorbance of the *Standard solution*

B_S = absorbance of the *Standard blank*

C_S = concentration of USP Methenamine RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of methenamine in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Procedure for a pooled sample

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Analysis: Determine the amount of methenamine ($C_6H_{12}N_4$) dissolved by using the procedure set forth in the *Assay*, making any necessary modifications.

Tolerances: NLT 75% (Q) of the labeled amount of methenamine ($C_6H_{12}N_4$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

ADDITIONAL REQUIREMENTS

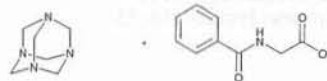
• PACKAGING AND STORAGE:

Preserve in well-closed containers.

• USP REFERENCE STANDARDS (11)

USP Methenamine RS

Methenamine Hippurate



$C_6H_{12}N_4 \cdot C_9H_9NO_3$ 319.36

Glycine, *N*-benzoyl, compd. with 1,3,5,7-tetraazatricyclo [3.3.1.1^{3,7}]decane (1:1);

Hexamethylenetetramine monohippurate [5714-73-8].

DEFINITION

Methenamine Hippurate, dried under vacuum at 60° for 1 h, contains NLT 95.5% and NMT 102.0% of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$), and contains NLT 54.0% and NMT 58.0% of hippuric acid ($C_9H_9NO_3$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197M)

ASSAY

• PROCEDURE

Sample solution: Dissolve 700 mg of Methenamine Hippurate in 50 mL of glacial acetic acid.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Titrate with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.94 mg of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$).

Acceptance criteria: 95.5%–102.0% on the previously dried basis

OTHER COMPONENTS

• CONTENT OF HIPPURIC ACID

Sample solution: Transfer 1 g to a 250-mL conical flask, and add 50 mL of water. When the solution is complete, add phenolphthalein TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium hydroxide VS

Endpoint detection: Visual

Analysis: Titrate with *Titrant*. Each mL of 0.1 N sodium hydroxide is equivalent to 17.92 mg of hippuric acid ($C_9H_9NO_3$).

Acceptance criteria: 54.0%–58.0%

IMPURITIES

• RESIDUE ON IGNITION (281):

NMT 0.1%

• SULFATE

Sample solution: 200 mg in 10 mL of water

Analysis: Add 5 drops of 3 N hydrochloric acid and 5 drops of barium chloride TS.

Acceptance criteria: No turbidity appears within 1 min.

Delete the following:

• HEAVY METALS, Method II (231):

NMT 15 ppm • (Official 1-

Jan-2018)

SPECIFIC TESTS

- **LOSS ON DRYING** (731)
Analysis: Dry under vacuum at 60° for 1 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)
USP Methenamine Hippurate RS

Methenamine Hippurate Tablets**DEFINITION**

Methenamine Hippurate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
Sample: A portion of finely powdered Tablets
Acceptance criteria: Meet the requirements

ASSAY• **PROCEDURE**

Sample solution: Transfer an equivalent to 700 mg of methenamine hippurate from finely powdered Tablets (NLT 20) to a 250-mL conical flask. Add 50 mL of alcohol, then add thymolphthalein TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N sodium hydroxide VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with *Titrant*. Perform a blank determination on a mixture of 50 mL of alcohol and 20 mL of water, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 31.94 mg of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$).

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)**Test 1**

Medium: Water; 900 mL

Apparatus 2: 100 rpm

Time: 30 min

Standard solution: 22 µg/mL of USP Methenamine Hippurate RS

Instrumental conditions

Mode: UV

Analytical wavelength: Maxima at about 227 nm

Analysis: Determine the quantity of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$) dissolved of filtered portions of the solution under test, suitably diluted with water if necessary, in comparison with the *Standard solution*.

Tolerances: NLT 80% (Q) of the labeled amount of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$) is dissolved.

Test 2

[NOTE—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.]

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Standard solution: 22 µg/mL of USP Methenamine Hippurate RS

Instrumental conditions

Mode: UV

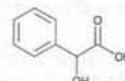
Analytical wavelength: Maxima at about 227 nm
Analysis: Determine the quantity of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$) dissolved of filtered portions of the solution under test, suitably diluted with water if necessary, in comparison with the *Standard solution*.

Tolerances: NLT 80% (Q) of the labeled amount of methenamine hippurate ($C_6H_{12}N_4 \cdot C_9H_9NO_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)
USP Methenamine Hippurate RS

Methenamine Mandelate

$C_6H_{12}N_4 \cdot C_8H_8O_3$ 292.33
Benzenecetic acid, α -hydroxy-, (\pm)-, compd. with 1,3,5,7-tetraazatricyclo[3.3.1.1^{3,7}]decane (1:1);
Hexamethylenetetramine mono-(\pm)-mandelate [587-23-5].

DEFINITION

Methenamine Mandelate contains NLT 95.5% and NMT 102.0% of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$), and contains NLT 50.0% and NMT 53.0% of mandelic acid ($C_8H_8O_3$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

ASSAY• **PROCEDURE**

Sample solution: Transfer 60 mg of Methenamine Mandelate to a 250-mL conical flask. Add 15 mL of dehydrated alcohol, stir to dissolve, and add 40 mL of chloroform.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 N silver nitrate in dehydrated alcohol prepared as follows. Dissolve by stirring 8.5 g of silver nitrate in 1000 mL of dehydrated alcohol. Transfer 100 mg of sodium chloride, previously dried at 110° for 2 h, to a 100-mL beaker, and dissolve in 50 mL of water. Titrate with the silver nitrate solution to the potentiometric endpoint, using a silver billet indicator electrode and a silver-silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Calculate the normality of the titrant.

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with *Titrant*, determining the endpoint potentiometrically, using a silver billet indicator electrode and a silver-silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 7.308 mg of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$).

Acceptance criteria: 95.5%–102.0% on the dried basis

OTHER COMPONENTS

• CONTENT OF MANDELIC ACID

Sample solution: Transfer 90 mg to a 250-mL conical flask containing 50 mL of water. When the solution is complete, titrate the magnetically stirred solution.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 N ceric ammonium nitrate VS

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with *Titrant*, determining the endpoint potentiometrically. Each mL of 0.05 N ceric ammonium nitrate is equivalent to 3.804 mg of mandelic acid ($C_8H_8O_3$).

Acceptance criteria: 50.0%–53.0% on the dried basis

IMPURITIES

• RESIDUE ON IGNITION (281): NMT 0.1%

• CHLORIDE AND SULFATE, Chloride (221)

Sample: 1.0 g

Analysis: Dissolve the *Sample* in 10 mL of water, and add gradually 500 mg of anhydrous sodium carbonate. Evaporate to dryness, and ignite the residue at a dull-red heat. Add 20 mL of 2 N nitric acid, stir gently, and filter.

Acceptance criteria: 0.01%; the filtrate shows no more chloride than corresponds to 0.15 mL of 0.020 N hydrochloric acid.

• SULFATE

Sample: 0.20 g

Analysis: Dissolve the *Sample* in 10 mL of water. Add 5 drops of 3 N hydrochloric acid and 5 drops of barium chloride TS.

Acceptance criteria: No turbidity appears within 1 min.

Delete the following:

• HEAVY METALS (231)

Test preparation: Dissolve 1.3 g in 10 mL of water, add 2 mL of 3 N hydrochloric acid, and dilute with water to 25 mL.

Acceptance criteria: NMT 15 ppm (Official 1-Jan-2018)

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry over silica gel for 18 h.

Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in well-closed containers.

• USP REFERENCE STANDARDS (11)

USP Methenamine Mandelate RS

Methenamine Mandelate for Oral Solution

DEFINITION

Methenamine Mandelate for Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197)

Sample: A finely powdered portion, equivalent to 100 mg of methenamine mandelate

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelenths as

that of a potassium bromide dispersion of USP Methenamine Mandelate RS.

ASSAY

• PROCEDURE

Sample solution: Accurately weigh the contents of NLT 10 containers of Methenamine Mandelate for Oral Solution, and reduce to a fine powder. Transfer an equivalent to 60 mg of methenamine mandelate from the powder to a 150-mL beaker. Add 15 mL of dehydrated alcohol, stir to dissolve, and add 40 mL of chloroform.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 N silver nitrate in dehydrated alcohol prepared as follows. Dissolve by stirring 8.5 g of silver nitrate in 1000 mL of dehydrated alcohol. Transfer 100 mg of sodium chloride, previously dried at 110° for 2 h, to a 100-mL beaker, and dissolve in 50 mL of water. Titrate with the silver nitrate solution to the potentiometric endpoint, using a silver billet indicator electrode and a silver–silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Calculate the normality of the titrant.

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with *Titrant*, determining the endpoint potentiometrically, using a silver billet indicator electrode and a silver–silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 7.308 mg of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$).

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• UNIFORMITY OF DOSAGE UNITS (905): Meets the requirements for powder packaged in single-unit containers

• DELIVERABLE VOLUME (698): Meets the requirements for powder packaged in multiple-unit containers

SPECIFIC TESTS

• pH (791)

Sample solution: 1 g in 30 mL of water

Acceptance criteria: 4.0–4.5

• WATER DETERMINATION, Method I (921): NMT 0.5%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in well-closed containers.

• LABELING: Label Methenamine Mandelate for Oral Solution that contains insoluble ingredients to indicate that the aqueous constituted Oral Solution contains dissolved methenamine mandelate but may remain turbid because of the presence of added substances.

• USP REFERENCE STANDARDS (11)

USP Methenamine Mandelate RS

Methenamine Mandelate Oral Suspension

DEFINITION

Methenamine Mandelate Oral Suspension is Methenamine Mandelate suspended in vegetable oil. It contains NLT 90.0% and NMT 110.0% of the labeled amount of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197)

Sample solution: Triturate the equivalent to 100 mg of methenamine mandelate, with 10 mL of chloroform, and pass through a 0.45- μ m pore size membrane filter.

Analysis: Evaporate the solvent in the *Sample solution*, wash the residue with five small portions of ether, and allow it to air-dry.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelenths as that of a potassium bromide dispersion of USP Methenamine Mandelate RS.

ASSAY

• PROCEDURE

Sample solution: Shake the Oral Suspension, then pipet, using a "to contain" pipet, an amount equivalent to 1 g of methenamine mandelate, into a 100-mL volumetric flask. Add 5.0 mL of dehydrated alcohol, mix, and add methylene chloride to volume. Pipet 5 mL of this solution into a 250-mL conical flask. Add 15 mL of dehydrated alcohol, and add 40 mL of chloroform.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 N silver nitrate in dehydrated alcohol prepared as follows. Dissolve by stirring 8.5 g of silver nitrate in 1000 mL of dehydrated alcohol. Transfer 100 mg of sodium chloride, previously dried at 110° for 2 h, to a 100-mL beaker, and dissolve in 50 mL of water. Titrate with the silver nitrate solution to the potentiometric endpoint, using a silver billet indicator electrode and a silver-silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Calculate the normality of the titrant.

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with *Titrant*, determining the endpoint potentiometrically, using a silver billet indicator electrode and a silver-silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 7.308 mg of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$).

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements for Oral Suspension packaged in single-unit containers
- **DELIVERABLE VOLUME (698):** Meets the requirements for Oral Suspension packaged in multiple-unit containers

SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 0.1%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Methenamine Mandelate RS

Methenamine Mandelate Tablets

DEFINITION

Methenamine Mandelate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$).

IDENTIFICATION

- **A. INFRARED ABSORPTION (197)**

Sample: Triturate a portion of finely powdered Tablets, equivalent to 5.0 mg of methenamine mandelate, with 5 mL of chloroform, and pass through a 0.45- μ m membrane filter. Evaporate the solvent, and allow the residue to air-dry.

Acceptance criteria: Meet the requirements

ASSAY

• PROCEDURE

Sample solution: Transfer an equivalent to 60 mg of methenamine mandelate, from NLT 20 finely powdered Tablets, to a 250-mL conical flask. Add 15 mL of dehydrated alcohol, stir to dissolve, and add 40 mL of chloroform.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 N silver nitrate in dehydrated alcohol prepared as follows. Dissolve by stirring 8.5 g of silver nitrate in 1000 mL of dehydrated alcohol. Transfer 100 mg of sodium chloride, previously dried at 110° for 2 h, to a 100-mL beaker, and dissolve in 50 mL of water. Titrate with the silver nitrate solution to the potentiometric endpoint, using a silver billet indicator electrode and a silver-silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Calculate the normality of the titrant.

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with *Titrant*, determining the endpoint potentiometrically, using a silver billet indicator electrode and a silver-silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 7.308 mg of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$).

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

For uncoated or plain coated Tablets

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: USP Methenamine Mandelate RS in *Medium*

Instrumental conditions

Mode: UV

Analytical wavelength: UV maximum at about 257 nm

Analysis: Determine the amount of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$) dissolved in the portion of the solution under test, passed through a filter of 0.45- μ m pore size and suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Methenamine Mandelate RS in the same *Medium*.

Tolerances: NLT 75% (Q) of the labeled amount of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
USP Methenamine Mandelate RS

Methenamine Mandelate Delayed-Release Tablets

DEFINITION

Methenamine Mandelate Delayed-Release Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$).

IDENTIFICATION• **A. INFRARED ABSORPTION (197K)**

Sample: Triturate an equivalent to 5.0 mg of methenamine mandelate from finely powdered Tablets with 5 mL of chloroform, and pass through a filter of 0.45- μ m pore size. Evaporate the solvent, and allow the residue to air-dry.

Acceptance criteria: Meet the requirements

ASSAY• **PROCEDURE**

Sample solution: Transfer an equivalent to 60 mg of methenamine mandelate, from finely powdered Tablets (NLT 20), to a 250-mL conical flask. Add 15 mL of dehydrated alcohol, stir to dissolve, and add 40 mL of chloroform.

Titrimetric system

Mode: Direct titration

Titrant: 0.05 N silver nitrate in dehydrated alcohol prepared as follows. Dissolve by stirring 8.5 g of silver nitrate in 1000 mL of dehydrated alcohol. Transfer 100 mg of sodium chloride, previously dried at 110° for 2 h, to a 100-mL beaker, and dissolve in 50 mL of water. Titrate with the silver nitrate solution to the potentiometric endpoint, using a silver billet indicator electrode and a silver-silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Calculate the normality of the titrant.

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with *Titrant*, determining the endpoint potentiometrically, using a silver billet indicator electrode and a silver-silver chloride double-junction reference electrode containing a potassium nitrate salt bridge. Each mL of 0.05 N silver nitrate is equivalent to 7.308 mg of methenamine mandelate ($C_6H_{12}N_4 \cdot C_8H_8O_3$).

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISINTEGRATION (701):** 2.5 h, determined as directed in *Delayed-Release (Enteric-Coated) Tablets*
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
USP Methenamine Mandelate RS

Methimazole

$C_4H_6N_2S$

114.17

2H-Imidazole-2-thione, 1,3-dihydro-1-methyl-; 1-Methylimidazole-2-thiol [60-56-0].

DEFINITION

Methimazole contains NLT 98.0% and NMT 101.0% of methimazole ($C_4H_6N_2S$), calculated on the dried basis.

IDENTIFICATION• **A. INFRARED ABSORPTION (197K)****ASSAY**• **PROCEDURE**

Sample: 250 mg of Methimazole

Analysis: Dissolve the *Sample* in 75 mL of water. Add 15.0 mL of 0.1 N sodium hydroxide VS, mix, and add,

with stirring, 30 mL of 0.1 N silver nitrate. Continue the titration with the 0.1 N sodium hydroxide VS, determining the end-point potentiometrically. Each mL of 0.1 N sodium hydroxide is equivalent to 11.42 mg of methimazole ($C_4H_6N_2S$).

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES• **RESIDUE ON IGNITION (281):** NMT 0.1%• **SELENIUM (291)**

Sample: 200 mg of Methimazole

Acceptance criteria: NMT 30 ppm

• **ORGANIC IMPURITIES**

Standard solution: 0.01 mg/mL each of USP

Methimazole RS, USP Methimazole Related Compound A RS, 1-methylimidazole, and USP Methimazole Related Compound C RS in chloroform

Sample solution: 10 mg/mL of Methimazole in chloroform

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m \times 0.25-mm; 0.5- μ m coating of G27

Temperatures

Injection port: 150°

Detector: 250°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	—	100	2
100	30	250	15

Carrier gas: Helium

Flow rate: 1.5 mL/min

Injection volume: 1 μ L

Injection type: Split ratio, 3:20

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between methimazole related compound A and 1-methylimidazole

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methimazole related compound A, 1-methylimidazole, and methimazole related compound C in the portion of Methimazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each related compound from the *Sample solution*

r_S = peak response of the corresponding related compound from the *Standard solution*

C_S = concentration of the corresponding related compound in the *Standard solution* (mg/mL)

C_U = concentration of Methimazole in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Methimazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of methimazole from the *Standard solution*

C_S = concentration of USP Methimazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Methimazole in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard any peak below 0.02%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methimazole related compound A	0.3	0.1
1-Methylimidazole	0.4	0.1
Methimazole related compound C	0.7	0.1
Methimazole	1.0	—
Any other individual impurity	—	0.1
Total impurities	—	0.5

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in well-closed, light-resistant containers.

• USP REFERENCE STANDARDS (11)

USP Methimazole RS

USP Methimazole Related Compound A RS

2,2-Dimethoxy-*N*-methylethanamine.

$C_5H_{13}NO_2$ 119.16

USP Methimazole Related Compound C RS

1-Methyl-2-(methylthio)-1*H*-imidazole.

$C_5H_8N_2S$ 128.20

Methimazole Tablets

DEFINITION

Methimazole Tablets contain NLT 94.0% and NMT 106.0% of the labeled amount of methimazole ($C_4H_6N_2S$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

Sample: Digest a quantity of powdered Tablets, equivalent to 10 mg of methimazole, with 10 mL of warm chloroform for 20 min, filter, and evaporate the filtrate on a steam bath to dryness.

Acceptance criteria: Meet the requirements

ASSAY

• PROCEDURE

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 120 mg of methimazole, to a 100-mL volumetric flask. Add about 80 mL of water, insert the stopper, and shake by mechanical means or occasionally by hand for 30 min. Dilute with water to volume, and filter.

Analysis: Add 3.5 mL of 0.1 N sodium hydroxide VS to 50.0 mL of *Sample solution*, mix, and add, with stirring, 7 mL of 0.1 N silver nitrate. Continue the titration with the 0.1 N sodium hydroxide VS, determining the end-point potentiometrically. Each mL of 0.1 N sodium hydroxide is equivalent to 11.42 mg of methimazole ($C_4H_6N_2S$).

Acceptance criteria: 94.0%–106.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: Water; 500 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: USP Methimazole RS at a known concentration in *Medium*

Sample solutions: Filtered solution under test, suitably diluted with *Medium*

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 252 nm

Tolerances: NLT 80% (Q) of the labeled amount of methimazole ($C_4H_6N_2S$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

Procedure for content uniformity

Standard solution: 5 µg/mL of USP Methimazole RS in water

Sample stock solution: Place 1 Tablet, previously crushed or finely powdered, in a 100-mL volumetric flask. Add 50 mL of water, and shake by mechanical means for 30 min. Dilute with water to volume, mix, and filter, discarding the first 20 mL of filtrate.

Sample solution: Nominally 5 µg/mL of methimazole in water from *Sample stock solution*

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 252 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of the labeled amount of methimazole ($C_4H_6N_2S$) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Methimazole RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of methimazole in the *Sample solution* (µg/mL)

ADDITIONAL REQUIREMENTS

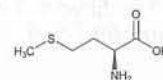
• PACKAGING AND STORAGE:

Preserve in well-closed, light-resistant containers.

• USP REFERENCE STANDARDS (11)

USP Methimazole RS

Methionine



$C_5H_{11}NO_2S$

L-Methionine [63-68-3].

149.21

DEFINITION

Methionine contains NLT 98.5% and NMT 101.5% of L-methionine ($C_5H_{11}NO_2S$), calculated on the dried basis.

IDENTIFICATION• **A. INFRARED ABSORPTION** (197K)**ASSAY**• **PROCEDURE**

Sample: 140 mg of Methionine

Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with the *Titrant*.

Calculate the percentage of methionine ($C_5H_{11}NO_2S$) in the *Sample* taken:

$$\text{Result} = \left[\frac{(V_S - V_B) \times N \times F}{W} \right] \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 149.2 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES• **RESIDUE ON IGNITION** (281): NMT 0.4%• **CHLORIDE AND SULFATE, Chloride** (221)

Standard solution: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.73 g of Methionine

Acceptance criteria: NMT 0.05%

• **CHLORIDE AND SULFATE, Sulfate** (221)

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.33 g of Methionine

Acceptance criteria: NMT 0.03 %

• **IRON** (241): NMT 30 ppm**Delete the following:**• **HEAVY METALS, Method I** (231): NMT 15 ppm (Official 1-

Jan-2018)

• **RELATED COMPOUNDS**

System suitability solution: 0.4 mg/mL each of USP L-Methionine RS and USP L-Serine RS in 0.3 N hydrochloric acid

Standard solution: 0.05 mg/mL of USP L-Methionine RS in 0.3 N hydrochloric acid. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample* solution.]

Sample solution: 10 mg/mL of Methionine in 0.3 N hydrochloric acid

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)

Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

System suitability

Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

Analysis

Samples: *System suitability solution*, *Standard solution*, and *Sample solution*

After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.

Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS• **OPTICAL ROTATION, Specific Rotation** (781S)

Sample solution: 20 mg/mL in 6 N hydrochloric acid

Acceptance criteria: +22.4° to +24.7°

• **pH** (791)

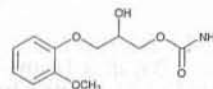
Sample solution: 10 mg/mL of solution

Acceptance criteria: 5.6–6.1

• **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h; it loses NMT 0.3% of its weight.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers.• **USP REFERENCE STANDARDS** (11)

USP L-Methionine RS

USP L-Serine RS

Methocarbamol

$C_{11}H_{15}NO_5$ 241.24
1,2-Propanediol, 3-(2-methoxyphenoxy)-, 1-carbamate, (±)-;
(±)-3-(o-Methoxyphenoxy)-1,2-propanediol 1-carbamate
[532-03-6].

DEFINITION

Methocarbamol contains NLT 98.5% and NMT 101.5% of methocarbamol ($C_{11}H_{15}NO_5$), calculated on the dried basis.

IDENTIFICATION• **A. INFRARED ABSORPTION** (197K)• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**ASSAY**• **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid or sodium hydroxide to a pH of 4.5.

Mobile phase: Methanol and *Buffer* (30:70)

System suitability solution: 1.0 mg/mL of USP Methocarbamol RS and 0.005 mg/mL of USP Guaifenesin RS in *Mobile phase*

Standard solution: 0.1 mg/mL of USP Methocarbamol RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Methocarbamol in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 274 nm

Column: 4.6-mm \times 15-cm; 3- μ m packing L1

Column temperature: 30°

Flow rate: 0.8 mL/min

Injection volume: 20 μ L

Run time: 1.5 times the retention time of methocarbamol

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.5 between methocarbamol and guaifenesin, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methocarbamol ($C_{11}H_{15}NO_5$) in the portion of Methocarbamol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methocarbamol from the *Sample solution*

r_S = peak response of methocarbamol from the *Standard solution*

C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)

C_U = concentration of Methocarbamol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

Delete the following:

- **HEAVY METALS (231), Method I**

Sample solution: 1.0 g in a 10-mL mixture of methanol and 1 N acetic acid (7:3), diluted with water to 25 mL

Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.005 mg/mL of USP Methocarbamol RS in *Mobile phase*

Sample solution: 1 mg/mL of Methocarbamol in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.5 between methocarbamol and guaifenesin, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Methocarbamol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of methocarbamol from the *Standard solution*

C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)

C_U = concentration of Methocarbamol in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Guaifenesin	0.84	1.2	0.15
Methocarbamol isomer ^a	0.90	1.0	0.05
Methocarbamol	1.0	—	—
Methocarbamol dioxolone ^b	1.3	1.0	0.05
Any individual unspecified impurity	—	—	0.05
Total impurities	—	—	1.0

^a 1-Hydroxy-3-(2-methoxyphenoxy)propan-2-yl carbamate.

^b 4-[(2-Methoxyphenoxy)methyl]-1,3-dioxolan-2-one.

SPECIFIC TESTS

- **LOSS ON DRYING (731)**

Analysis: Dry at 60° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

- **USP REFERENCE STANDARDS (11)**

USP Guaifenesin RS

USP Methocarbamol RS

Methocarbamol Injection**DEFINITION**

Methocarbamol Injection is a sterile solution of Methocarbamol in an aqueous solution of Polyethylene Glycol 300. It contains NLT 95.0% and NMT 105.0% of the labeled amount of methocarbamol ($C_{11}H_{15}NO_5$).

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

Sample: Mix a volume with 40 mL of water equivalent to 500 mg of methocarbamol from Injection in a small separator. Extract with 10 mL of ethyl acetate, and dry the ethyl acetate layer over anhydrous sodium sulfate. Evaporate the ethyl acetate with the use of a water bath maintained at 40° under a stream of nitrogen to dryness.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of 4.5.

Mobile phase: Methanol and *Buffer* (30:70)

Standard solution: 1 mg/mL of USP Methocarbamol RS in *Mobile phase*

Sample solution: Nominally 1 mg/mL of methocarbamol from a suitable volume of Injection containing NLT 100 mg of methocarbamol in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 274 nm

Column: 4.6-mm × 10.0-cm; 3-μm or 5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methocarbamol ($C_{11}H_{15}NO_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methocarbamol in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

IMPURITIES

• LIMIT OF ALDEHYDES

Diluent: Alcohol and water (20:80)

Solution A: 10 mg/mL of phenylhydrazine hydrochloride in *Diluent*

Solution B: 10 mg/mL of potassium ferricyanide in water

Solution C: 10 µg/mL of formaldehyde in water prepared as follows. Dissolve 1.37 g of formaldehyde solution in 1 L of water. Dilute 10 mL of the resulting solution with water to 500 mL.

Standard solution: Transfer 4 mL of *Solution C* to a 25-mL volumetric flask. Add 2.0 mL of filtered *Solution A*. Allow to stand for 10 min. Add 1 mL of *Solution B*, and allow to stand for 5 min. Add 4 mL of hydrochloric acid, and dilute with alcohol to volume.

Sample solution: Empty the contents of NLT 10 vials of Injection to a suitable container. Transfer 4.0 mL of the composite sample of Injection to a 25-mL volumetric flask. Add 2.0 mL of filtered *Solution A*, and allow to stand for 10 min. Add 1 mL of *Solution B*, and allow to stand for 5 min. Add 4 mL of hydrochloric acid, and dilute with alcohol to volume.

Blank: Transfer 4 mL of water to a 25-mL volumetric flask. Add 2.0 mL of filtered *Solution A*, and allow to stand for 10 min. Add 1 mL of *Solution B*, and allow to stand for 5 min. Add 4 mL of hydrochloric acid, and dilute with alcohol to volume.

Instrumental conditions

Mode: Vis

Analytical wavelength: 515 nm

Cell: 1 cm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Determine the absorbances of the *Samples*.

Acceptance criteria: The absorbance of the *Sample solution* is NMT the absorbance of the *Standard solution* (NMT 10 µg of formaldehyde in each mL of Injection).

SPECIFIC TESTS

• **pH (791):** 3.5–6.0

• **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.2 USP Endotoxin Units/mg of methocarbamol

• **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections

• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers. Store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Endotoxin RS

USP Methocarbamol RS

Methocarbamol Tablets

DEFINITION

Methocarbamol Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of methocarbamol ($C_{11}H_{15}NO_3$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

Sample: Mix a portion of finely powdered Tablets equivalent to 1 g of methocarbamol with 25 mL of water in a separator, and extract with 25 mL of chloroform. Filter the extract, and evaporate to dryness.

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid or sodium hydroxide to a pH of 4.5.

Mobile phase: Methanol and *Buffer* (30:70)

System suitability solution: 1.0 mg/mL of USP Methocarbamol RS and 0.005 mg/mL of USP Guaifenesin RS in *Mobile phase*

Standard solution: 0.1 mg/mL of USP Methocarbamol RS in *Mobile phase*

Sample stock solution: Nominally 1 mg/mL of methocarbamol solution prepared as follows. Transfer a portion of finely powdered Tablets (NLT 10) to a volumetric flask of suitable size. Add 60% of the volume of the flask with *Mobile phase*. Sonicate for 30 min with intermittent shaking. Dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Sample solution: Nominally 0.1 mg/mL of methocarbamol from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 274 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Column temperature: 30°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

Run time: 1.5 times the retention time of methocarbamol

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 3.5 between methocarbamol and guaifenesin, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methocarbamol ($C_{11}H_{15}NO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of methocarbamol from the *Sample solution*
 r_S = peak response of methocarbamol from the *Standard solution*
 C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of methocarbamol in the *Sample solution* (mg/mL)
 Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS**• DISSOLUTION (711)**

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Standard solution: USP Methocarbamol RS in Medium

Sample solution: Filtered portion of the solution under test, diluted with Medium if necessary

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methocarbamol ($C_{11}H_{15}NO_3$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

- r_U = peak response of methocarbamol from the *Sample solution*
 r_S = peak response of methocarbamol from the *Standard solution*
 C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)
 V = volume of Medium, 900 mL
 L = label claim for methocarbamol (mg/Tablet)
 Tolerances: NLT 75% (Q) of the labeled amount of methocarbamol ($C_{11}H_{15}NO_3$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

IMPURITIES

Change to read:

• ORGANIC IMPURITIES

Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 0.005 mg/mL of USP Methocarbamol RS in *Mobile phase*

Sample solution: Use the *Sample stock solution* from the Assay.

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See Table 1 for relative retention times.]

Suitability requirements

Resolution: NLT 3.5 between methocarbamol and guaifenesin, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- r_U = peak response of each degradation product from the *Sample solution*
 r_S = peak response of methocarbamol from the *Standard solution*
 C_S = concentration of USP Methocarbamol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methocarbamol in the *Sample solution* (mg/mL)

F = relative response factor (see Table 1)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Guaifenesin	0.84	1.2	0.15
Methocarbamol isomer ^a	0.90	1.0	0.05
Methocarbamol	1.0	—	—
Methocarbamol dioxolone ^b	1.3	1.0	0.05
Any individual unspecified degradation product	—	—	0.10
Total impurities	—	—	1.0

^a 1-Hydroxy-3-(2-methoxyphenoxy)propan-2-yl carbamate.

^b 4-[(2-Methoxyphenoxy)methyl]-1,3-dioxolan-2-one.

• (RB 1-Apr-2016)

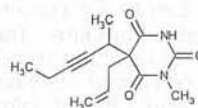
ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in tight containers. Store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Guaifenesin RS

USP Methocarbamol RS

Methohexital

$C_{14}H_{18}N_2O_3$ 262.30

2,4,6-(1*H*,3*H*,5*H*)-Pyrimidinetrione, 1-methyl-5-(1-methyl-2-pentenyl)-5-(2-propenyl)-, (±)-.

(±)-5-Allyl-1-methyl-5-(1-methyl-2-pentenyl)barbituric acid [151-83-7].

» Methohexital contains not less than 98.0 percent and not more than 101.0 percent of $C_{14}H_{18}N_2O_3$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Methohexital RS

Identification, Infrared Absorption (197S)—

Solution: 1 in 100.

Medium: chloroform.

Melting range (741): between 92° and 96°, but the range between beginning and end of melting does not exceed 3°.

Water Determination, Method I (921): not more than 2.0%.

Chloride (221)—Dissolve 200 mg in a mixture of 75 mL of ether and 25 mL of water, agitate, and allow to separate: the water solution shows no more chloride than corresponds to 0.17 mL of 0.010 N hydrochloric acid (0.03%).

Delete the following:

• **Heavy metals, Method II (231):** 0.001%. • (Official 1-Jan-2018)

Ordinary impurities (466)—

Test solution: methanol.

Standard solution: methanol.

Eluent: a mixture of chloroform and acetone (7:3).

Visualization—Expose the plate to chlorine gas for 1 minute, and air-dry the plate at room temperature for 2 minutes. Prepare a solution of 0.5 g of potassium iodide in 50 mL of water, and prepare a solution of 1.5 g of soluble starch in 50 mL of hot water. Mix 10 mL of each solution with 4 mL of alcohol to obtain the *Detection reagent*.

[NOTE—The *Detection reagent* so obtained may be used for up to 3 or 4 days.] Spray the plate with the *Detection reagent*.

Assay—Dissolve about 100 mg of Methohexital, accurately weighed, in chloroform, and dilute quantitatively and stepwise with chloroform to obtain a solution having a concentration of about 10 mg per mL. Dissolve an accurately weighed quantity of USP Methohexital RS in chloroform, and dilute quantitatively and stepwise with chloroform to obtain a Standard solution having a known concentration of about 10 mg per mL. Concomitantly determine the absorbances of both solutions in 0.1-mm cells at the wavelength of maximum absorbance at about 5.93 μm , with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_3$ in the portion of Methohexital taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Methohexital RS in the Standard solution; and A_U and A_S are the absorbances of the solution of Methohexital and the Standard solution, respectively.

Methohexital Sodium for Injection

$\text{C}_{14}\text{H}_{17}\text{N}_2\text{NaO}_3$ 284.29

2,4,6-(1*H*,3*H*,5*H*)-Pyrimidinetrione, 1-methyl-5-(1-methyl-2-pentynyl)-5-(2-propenyl)-, (\pm)-, monosodium salt. Sodium 5-allyl-1-methyl-5-(1-methyl-2-pentynyl)barbiturate [309-36-4; 22151-68-4].

» Methohexital Sodium for Injection is a freeze-dried, sterile mixture of methohexital sodium and anhydrous Sodium Carbonate as a buffer, prepared from an aqueous solution of Methohexital, Sodium Hydroxide, and Sodium Carbonate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methohexital sodium ($\text{C}_{14}\text{H}_{17}\text{N}_2\text{NaO}_3$).

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Store at controlled room temperature. Injection may be packaged in 50-mL multiple-dose containers.

USP Reference standards (11)—

USP Endotoxin RS

USP Methohexital RS

Completeness of solution—Mix 1 g with 20 mL of carbon dioxide-free water: after 1 minute, the solution is clear and free from undissolved solid.

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products (Parenterals)*—*Product Quality Tests* (1), *Specific Tests, Completeness and clarity of solutions*.

Identification—

A: Dissolve about 500 mg in 10 mL of water in a separator, add 10 mL of 3 N hydrochloric acid, and extract the liberated methohexital with two 25-mL portions of chloroform. Evaporate the combined chloroform extracts to dryness, add 10 mL of ether, evaporate again, and dry the residue in vacuum at 80° for 4 hours. Dissolve 50 mg of the residue so obtained in 5 mL of chloroform: the solution exhibits IR absorption maxima at the same wavelengths as that of a similar preparation of USP Methohexital RS.

B: The methohexital obtained and dried as directed for *Identification test A* melts between 92° and 96°.

Bacterial Endotoxins Test (85)—It contains not more than 0.5 USP Endotoxin Unit per mg of methohexital sodium.

Uniformity of dosage units (905): meets the requirements.

Procedure for content uniformity—

Standard solution—Transfer about 23 mg of USP Methohexital RS, accurately weighed, to a 250-mL volumetric flask, add 50 mL of water, 0.5 mL of sodium hydroxide solution (1 in 10), and 1.5 mL of sodium carbonate solution (1 in 1000), and mix. Dilute with water to volume, and mix. Transfer 20.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

Test solution—Transfer the contents of 1 vial of Methohexital Sodium for Injection with the aid of water to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer an accurately measured volume of this solution, equivalent to about 100 mg of methohexital sodium, to a 1000-mL volumetric flask, add about 200 mL of water and 2.0 mL of sodium hydroxide solution (1 in 10), mix, dilute with water to volume, and again mix. Transfer 20.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the Standard solution and the Test solution in 1-cm cells at the wavelength of maximum absorbance at about 247 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $\text{C}_{14}\text{H}_{17}\text{N}_2\text{NaO}_3$ in the Methohexital Sodium for Injection taken by the formula:

$$(284.29/262.30)(TC/D)(A_U / A_S)$$

in which 284.29 and 262.30 are the molecular weights of methohexital sodium and methohexital, respectively; T is the labeled quantity, in mg, of methohexital sodium in the Methohexital Sodium for Injection; C is the concentration, in μg per mL, of USP Methohexital RS in the Standard solution; D is the concentration, in μg per mL, of methohexital sodium in the Test solution based on the labeled quantity per container and the extent of dilution; and A_U and A_S are the absorbances of the Test solution and the Standard solution, respectively.

pH (791): between 10.6 and 11.6 in the solution prepared in the test for *Completeness of solution*.

Loss on drying (731)—Dry it at 105° for 4 hours: it loses not more than 2.0% of its weight.

Delete the following:

• **Heavy metals, Method II (231):** 0.001%. • (Official 1-Jan-2018)

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Internal standard solution—Dissolve aprobarbital in chloroform to obtain a solution having a concentration of about 1.35 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Methohexital RS in chloroform to obtain a solution having a known concentration of about 0.46 mg

per mL. Transfer 5.0 mL of the resulting solution to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with chloroform to volume, and mix to obtain a *Standard preparation* having a known concentration of about 230 µg per mL.

Assay preparation—Combine and mix the constituted solutions prepared from the contents of 5 vials of Methohexital Sodium for Injection. Transfer an accurately measured volume of the resulting solution, equivalent to about 50 mg of methohexital sodium, to a 125-mL separator containing 25 mL of water, and mix. Add 0.2 mL of dilute hydrochloric acid (1 in 2), and mix. Extract with three 25-mL portions of chloroform, shaking each extraction for 2 minutes and filtering the extracts through about 15 g of anhydrous sodium sulfate, that previously has been washed with about 5 mL of chloroform, into a 100-mL volumetric flask. Wash the sodium sulfate with several small portions of chloroform, collecting the washings in the 100-mL volumetric flask. Dilute with chloroform to volume, and mix. Transfer 5.0 mL of this solution to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution*, dilute with chloroform to volume, and mix.

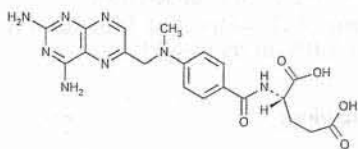
Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and contains a 1.2-m × 4-mm column packed with 3% phase G10 on support S1AB. The column is maintained at about 230°, the injection port at about 265°, and the detector block at about 265°. Dry helium is used as the carrier gas at a flow rate of about 60 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between methohexital and aprobarbital is not less than 4.0, and the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 2 µL) of the *Assay preparation* and the *Standard preparation* into the gas chromatograph, and measure the peak responses for the major peak. The relative retention times are about 0.6 for methohexital and 1.0 for aprobarbital. Calculate the quantity, in mg, of methohexital sodium (C₁₄H₁₇N₃NaO₃) in the portion of Methohexital Sodium for Injection taken by the formula:

$$(284.29/262.30)(0.2C)(R_u / R_s)$$

in which 284.29 and 262.30 are the molecular weights of methohexital sodium and methohexital, respectively; *C* is the concentration, in µg per mL, of USP Methohexital RS in the *Standard preparation*; and *R_u* and *R_s* are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methotrexate



C₂₀H₂₂N₈O₅

454.44

L-Glutamic acid, N-[4-[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-;

L-(+)-N-[p-[(2,4-Diamino-6-pteridinyl)methyl]methylamino]benzoyl] glutamic acid;

(S)-2-(4-[(2,4-Diaminopteridin-6-yl)methyl](methylamino)benzamido)pentanedioic acid [59-05-2].

DEFINITION

Methotrexate is a mixture of 4-amino-10-methylfolic acid and closely related compounds. It contains NLT 98.0% and NMT 102.0% of C₂₀H₂₂N₈O₅, calculated on the anhydrous basis.

[CAUTION]—Great care should be taken to prevent inhaling particles of Methotrexate and exposing the skin to it.]

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K): Do not dry specimens.
- **B. ULTRAVIOLET ABSORPTION** (197U)

Sample solution: 10 µg/mL in 0.1 N hydrochloric acid

ASSAY

• PROCEDURE

Buffer: 3.4 mg/mL of anhydrous monobasic sodium phosphate in water. Adjust with 1 N sodium hydroxide to a pH of 6.0.

Solution A: Acetonitrile and *Buffer* (1:19)

Solution B: Acetonitrile and *Buffer* (1:1)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	50	50
34	50	50
35	100	0
40	100	0

Standard stock solution: 1.0 mg/mL of USP Methotrexate RS prepared as follows. Transfer a known amount of USP Methotrexate RS to a suitable volumetric flask, dissolve in dimethyl sulfoxide equivalent to 5% of the final volume, and dilute with *Solution A* to volume.

Standard solution: 0.2 mg/mL of USP Methotrexate RS in *Solution A*, from the *Standard stock solution*

Sample stock solution: Transfer 200 mg of Methotrexate to a 200-mL volumetric flask, and dissolve in 10 mL of dimethyl sulfoxide with sonication for 5 min. Add 150 mL of *Solution A*, and sonicate again for 5 min. Dilute with *Solution A* to volume to obtain 1.0 mg/mL of Methotrexate. [NOTE—Sonicate as needed.]

Sample solution: 0.2 mg/mL of Methotrexate in *Solution A*, from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.6

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of C₂₀H₂₂N₈O₅ in the portion of Methotrexate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Methotrexate RS in the *Standard solution* (mg/mL)

C_u = concentration of Methotrexate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES**Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm (Official 1-Jan-2018)

Organic Impurities• **PROCEDURE 1: RELATED COMPOUNDS**

Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard stock solution A: Use the *Standard solution* from the Assay.

Standard solution A: 0.1 µg/mL of USP Methotrexate RS in *Solution A*, from *Standard stock solution A*

Standard stock solution B: Transfer known quantities of USP Methotrexate Related Compound C RS, USP Methotrexate Related Compound B RS, and USP Methotrexate Related Compound E RS to a suitable volumetric flask, dissolve in dimethyl sulfoxide equivalent to 1% of the final volume, and dilute with *Solution A* to volume to obtain 0.1 mg/mL of USP Methotrexate Related Compound B RS, 0.2 mg/mL of USP Methotrexate Related Compound C RS, and 0.1 mg/mL of USP Methotrexate Related Compound E RS.

Standard solution B: 0.4 µg/mL of USP Methotrexate Related Compound C RS, 0.2 µg/mL of USP Methotrexate Related Compound B RS, and 0.2 µg/mL of USP Methotrexate Related Compound E RS in *Solution A*, from *Standard stock solution B*

System suitability solution: Transfer a known quantity of USP Methotrexate System Suitability Mixture RS to a suitable volumetric flask, and dissolve in dimethyl sulfoxide equivalent to about 1% of the final volume. Add *Standard stock solution B* equivalent to 0.2% of the final volume, and dilute with *Solution A* to volume to prepare 0.1 mg/mL of USP Methotrexate System Suitability Mixture RS, 0.2 µg/mL of USP Methotrexate Related Compound B RS, 0.4 µg/mL of USP Methotrexate Related Compound C RS, and 0.2 µg/mL of USP Methotrexate Related Compound E RS.

System suitability

Samples: *Standard solution A* and *System suitability solution*

Suitability requirements

Resolution: NLT 1.7 between methotrexate related compound B and methotrexate related compound C, NLT 10.0 between methotrexate related compound C and methotrexate, and NLT 5.0 between methotrexate related compound I and methotrexate related compound H; *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of methotrexate related compound B and methotrexate related compound C in the portion of Methotrexate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from *Standard solution B*
 C_S = concentration of the corresponding methotrexate related compound in *Standard solution B* (mg/mL)
 C_U = concentration of Methotrexate in the *Sample solution* (mg/mL)

Calculate the percentage of methotrexate related compound E free base in the portion of Methotrexate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from *Standard solution B*
 C_S = concentration of USP Methotrexate Related Compound E RS in *Standard solution B* (mg/mL)
 C_U = concentration of Methotrexate in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of methotrexate related compound E free base, 325.33
 M_{r2} = molecular weight of USP Methotrexate Related Compound E RS, 343.56

[NOTE—USP Methotrexate Related Compound E RS is 4-[[[(2,4-diaminopteridin-6-yl)methyl](methyl)amino]benzoic acid, hemihydrochloride.]

Calculate the percentage of methotrexate related compound H, methotrexate related compound I, and any unspecified impurity in the portion of Methotrexate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response of methotrexate from *Standard solution A*
 C_S = concentration of USP Methotrexate RS in *Standard solution A* (mg/mL)
 C_U = concentration of Methotrexate in the *Sample solution* (mg/mL)
 F = relative response factor for each individual impurity (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*. [NOTE—Disregard any impurity peak less than 0.05%.]

Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Methotrexate related compound B ^a	0.71	—	0.3
Methotrexate related compound C ^b	0.75	—	0.5
Methotrexate	1.00	—	—
Methotrexate related compound E free base ^c	1.39	—	0.3
Methotrexate dimethylamide ^d and Methotrexate related compound I ^e	1.55	0.71	0.2 ^f

^a (S)-2-[(2,4-Diaminopteridin-6-yl)methylamino]benzamido]pentanedioic acid.

^b (S)-2-4-[[[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl)methyl](methyl)amino]benzamido]pentanedioic acid.

^c 4-[[[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino]benzoic acid.

^d 2-4-[[[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino]benzamido]-5-(dimethylamino)-5-oxopentanoic acid.

^e (S)-4-4-[[[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino]benzamido]-5-methoxy-5-oxopentanoic acid.

^f If present, methotrexate dimethylamide and Methotrexate related compound I may not be completely resolved by the method. These peaks are integrated together to determine conformance.

^g (S)-2-4-[[[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino]benzamido]-5-methoxy-5-oxopentanoic acid.

Impurity Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Methotrexate related compound H ^g	1.68	1.0	0.2
Any unspecified impurity	—	1.0	0.10

^a (S)-2-[4-[(2,4-Diaminopteridin-6-yl)methylamino]benzamido]pentanedioic acid.

^b (S)-2-[4-[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl)methyl](methylamino)benzamido]pentanedioic acid.

^c 4-[(2,4-Diaminopteridin-6-yl)methyl](methylamino)benzoic acid.

^d 2-[4-[(2,4-Diaminopteridin-6-yl)methyl](methylamino)benzamido]-5-(dimethylamino)-5-oxopentanoic acid.

^e (S)-4-[4-[(2,4-Diaminopteridin-6-yl)methyl](methylamino)benzamido]-5-methoxy-5-oxopentanoic acid.

^f If present, methotrexate dimethylamide and Methotrexate related compound I may not be completely resolved by the method. These peaks are integrated together to determine conformance.

^g (S)-2-[4-[(2,4-Diaminopteridin-6-yl)methyl](methylamino)benzamido]-5-methoxy-5-oxopentanoic acid.

• PROCEDURE 2: ENANTIOMERIC PURITY

Solution A: 7.1 g/L of anhydrous dibasic sodium phosphate in water

Solution B: 6.9 g/L of monobasic sodium phosphate in water

Solution C: *Solution A* and *Solution B* (5:6). Adjust with 2 N sodium hydroxide to a pH of 6.9.

Mobile phase: *n*-Propanol and *Solution C* (2:23)

System suitability solution: 0.02 mg/mL each of USP Methotrexate RS and USP *R*-Methotrexate RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Methotrexate in *Mobile phase*

Diluted sample solution: 2 µg/mL of Methotrexate in *Mobile phase*, from the *Sample solution*

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 302 nm

Column: 4.0-mm × 15-cm; 7-µm packing L75

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*. [NOTE—The relative retention times for methotrexate and *R*-methotrexate are 1.0 and 1.95, respectively.]

Suitability requirements

Resolution: NLT 1.3 between methotrexate and *R*-methotrexate

Relative standard deviation: NMT 5.0% for the methotrexate peak

Analysis

Samples: *Sample solution* and *Diluted sample solution*
Calculate the percentage of *R*-methotrexate in the portion of Methotrexate taken:

$$\text{Result} = [r_u/(r_s \times 100)] \times 100$$

r_u = peak area of *R*-methotrexate from the *Sample solution*

r_s = peak area of Methotrexate from the *Diluted sample solution*

Acceptance criteria: NMT 3.0%

SPECIFIC TESTS

• **WATER DETERMINATION, Method I** <921>: NMT 12.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• USP REFERENCE STANDARDS <11>

USP Methotrexate RS

USP Methotrexate Related Compound B RS

USP Methotrexate Related Compound C RS

USP Methotrexate Related Compound E RS

USP Methotrexate System Suitability Mixture RS

It contains Methotrexate, Methotrexate Dimethylester Hydrochloride

(S)-Dimethyl-2-[4-[(2,4-diaminopteridin-6-yl)methyl](methylamino)benzamido]pentanedioate hydrochloride.

$C_{22}H_{26}N_8O_5 \cdot HCl$ 518.95

and a small amount of Methotrexate related compound I

(S)-4-[4-[(2,4-Diaminopteridin-6-yl)methyl](methylamino)benzamido]-5-methoxy-5-oxopentanoic acid.

$C_{21}H_{24}N_8O_5$ 468.47

and Methotrexate related compound H

(S)-2-[4-[(2,4-Diaminopteridin-6-yl)methyl](methylamino)benzamido]-5-methoxy-5-oxopentanoic acid.

$C_{21}H_{24}N_8O_5$ 468.47

USP *R*-Methotrexate RS

(R)-2-[4-[(2,4-Diaminopteridin-6-yl)methyl](methylamino)benzamido]pentanedioic acid.

$C_{20}H_{22}N_8O_5$ 454.44

Methotrexate Injection

DEFINITION

Methotrexate Injection is a sterile solution of Methotrexate in Water for Injection prepared with the aid of Sodium Hydroxide. It contains NLT 90.0% and NMT 110.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$).

IDENTIFICATION

• A. INFRARED ABSORPTION <197K>

Sample: Dilute, if necessary, a volume of Injection, equivalent to about 25 mg of methotrexate, with water to obtain a solution having a concentration of about 2.5 mg/mL. Adjust with 0.1 N hydrochloric acid to a pH of 4.0. Place the slurry in a 50-mL centrifuge tube, and centrifuge. Decant the supernatant, add 25 mL of acetone, shake, and filter through a solvent-resistant membrane filter of 0.45-µm pore size. Air-dry the filtered precipitate.

Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Buffer: 0.2 M dibasic sodium phosphate and 0.1 M citric acid (63:37), adjusted if necessary with 0.1 M citric acid or 0.2 M dibasic sodium phosphate to a pH of 6.0

Mobile phase: Acetonitrile and *Buffer* (10:90)

System suitability solution: 0.1 mg/mL each of USP Methotrexate RS and folic acid in *Mobile phase*

Standard solution: 100 µg/mL of USP Methotrexate RS in *Mobile phase*

Sample solution: Equivalent to 100 µg/mL of methotrexate from Injection in *Mobile phase*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 302 nm**Column:** 4.6-mm × 25-cm; packing L1**Flow rate:** 1.2 mL/min**Injection volume:** 10 µL**System suitability****Sample:** *System suitability solution*

[NOTE—The relative retention times for folic acid and methotrexate are 0.35 and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 8.0 between the folic acid and methotrexate peaks**Relative standard deviation:** NMT 2.5% for the methotrexate peak**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount methotrexate ($C_{20}H_{22}N_8O_5$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Methotrexate RS in the *Standard solution* (µg/mL) C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)**Acceptance criteria:** 90.0%–110.0%**SPECIFIC TESTS**

- **PH (791):** 7.0–9.0
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.4 USP Endotoxin Unit/mg of methotrexate sodium
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections* (1)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.
- **USP REFERENCE STANDARDS (11)**
USP Endotoxin RS
USP Methotrexate RS

Methotrexate for Injection**DEFINITION**

Methotrexate for Injection is a sterile, freeze-dried preparation of methotrexate sodium with or without suitable added substances, buffers, and/or diluents. It contains NLT 95.0% and NMT 115.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$).

[CAUTION—Great care should be taken to prevent inhaling particles of methotrexate sodium and exposure to skin.]

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

Sample: 2.5 mg/mL of methotrexate in water from Methotrexate for Injection. Adjust with 0.1 N hydrochloric acid to a pH of 4.0. Place the slurry in a 50-mL centrifuge tube, and centrifuge. Decant the supernatant, add 25 mL of acetone, shake, and filter through a solvent-resistant membrane filter of 0.45-µm pore size. Air-dry the filtered precipitate.

Acceptance criteria: Meets the requirements**ASSAY**

- **PROCEDURE**

Buffer: 0.2 M dibasic sodium phosphate and 0.1 M citric acid (63:37), adjusted if necessary with 0.1 M citric acid or 0.2 M dibasic sodium phosphate to a pH of 6.0

Mobile phase: Acetonitrile and *Buffer* (10:90)**System suitability solution:** 0.1 mg/mL each of USP Methotrexate RS and folic acid in *Mobile phase***Standard solution:** 100 µg/mL of USP Methotrexate RS in *Mobile phase***Sample solution:** Equivalent to 100 µg/mL of methotrexate from 1 container of Methotrexate for Injection in *Mobile phase***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 302 nm**Column:** 4.6-mm × 25-cm; packing L1**Flow rate:** 1.2 mL/min**Injection volume:** 10 µL**System suitability****Sample:** *System suitability solution*

[NOTE—The relative retention times for folic acid and methotrexate are 0.35 and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 8.0 between the folic acid and methotrexate peaks**Relative standard deviation:** NMT 2.5% for the methotrexate peak**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$) in the portion of Methotrexate for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Methotrexate RS in the *Standard solution* (µg/mL) C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)**Acceptance criteria:** 95.0%–115.0%**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

SPECIFIC TESTS

- **PH (791)**

Sample: Constituted as directed in the labeling, except that water is used as the diluent

Acceptance criteria: 7.0–9.0

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.4 USP Endotoxin Unit/mg of methotrexate sodium
- **STERILITY TESTS (71):** Meets the requirements
- **OTHER REQUIREMENTS:** Meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*, protected from light.

• **USP REFERENCE STANDARDS** (11)

USP Endotoxin RS
USP Methotrexate RS

Methotrexate Tablets

DEFINITION

Methotrexate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$).

IDENTIFICATION

• **A. ULTRAVIOLET ABSORPTION** (197U)

Standard solution: 25 µg/mL of USP Methotrexate RS in dilute hydrochloric acid (1 in 100)

Sample solution: Dissolve 1 Tablet in 100 mL of dilute hydrochloric acid (1 in 100), and filter the solution.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*.

ASSAY

• **PROCEDURE**

Buffer: 0.2 M dibasic sodium phosphate and 0.1 M citric acid (63:37), adjusted if necessary with 0.1 M citric acid or 0.2 M dibasic sodium phosphate to a pH of 6.0

Mobile phase: Acetonitrile and *Buffer* (10:90)

System suitability solution: 0.1 mg/mL each of USP Methotrexate RS and folic acid in *Mobile phase*

Standard solution: 100 µg/mL of USP Methotrexate RS in *Mobile phase*

Sample solution: Equivalent to 100 µg/mL of methotrexate from powdered Tablets (NLT 20 Tablets) in *Mobile phase*. Dissolve the methotrexate using a mechanical shaker or ultrasonic bath.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 302 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for folic acid and methotrexate are 0.35 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8.0 between the folic acid and methotrexate peaks

Relative standard deviation: NMT 2.5% for the methotrexate peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methotrexate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: USP Methotrexate RS in *Medium*

Detector: UV 306 nm (maximum absorbance)

Analysis: Determine the amount of $C_{20}H_{22}N_8O_5$ dissolved from UV absorbances on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

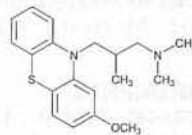
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. A unit-of-use container contains a quantity of Tablets sufficient to provide one week's therapy as indicated in the labeling.

• **LABELING:** When packaged in a unit-of-use container, the label indicates the total amount of methotrexate present as one week's supply.

• **USP REFERENCE STANDARDS** (11)
USP Methotrexate RS

Methotrimoprazine



$C_{19}H_{24}N_2OS$ 328.47
10*H*-Phenothiazine-10-propanamine, 2-methoxy-*N,N*,β-trimethyl-, (–)-;
(–)-10-[3-(Dimethylamino)-2-methylpropyl]-2-methoxyphenothiazine [60-99-1].

DEFINITION

Methotrimoprazine contains NLT 98.0% and NMT 101.0% of methotrimoprazine ($C_{19}H_{24}N_2OS$), calculated on the dried basis.

[NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and the solutions containing them by conducting the procedures without delay under subdued light or by using low-actinic glassware.]

IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

• **B. ULTRAVIOLET ABSORPTION** (197U)

Solution: 7 µg/mL

Medium: Alcohol

Acceptance criteria: Absorptivities at 255 nm, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY

• **PROCEDURE**

Sample solution: Dissolve about 700 mg of Methotrimoprazine in 100 mL of chloroform. Add 1 drop of a solution (1 in 500) of crystal violet in chloroform.

Analysis: Titrate the *Sample solution* with 0.1 N perchloric acid VS to the first disappearance of the violet tinge. Perform a blank determination, and make any necessary

correction. Each mL of 0.1 N perchloric acid is equivalent to 32.85 mg of methotrimeprazine ($C_{19}H_{24}N_2OS$).
Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

- **SELENIUM (291)**
Test solution: Prepare with 100 mg of Methotrimeprazine and 100 mg of magnesium oxide.
Acceptance criteria: The absorbance of the *Test solution* is NMT one-half that of the *Standard Solution* (0.003%).

SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation (781S)**
Sample solution: 50 mg/mL in chloroform
Acceptance criteria: -15° to -18°
- **LOSS ON DRYING (731)**
Analysis: Dry at 100° for 3 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at 25° , excursions permitted between 15° and 30° .
- **USP REFERENCE STANDARDS (11)**
 USP Methotrimeprazine RS

Methotrimeprazine Injection

DEFINITION

Methotrimeprazine Injection is a sterile solution of Methotrimeprazine in Water for Injection, prepared with the aid of hydrochloric acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of methotrimeprazine ($C_{19}H_{24}N_2OS$), as the hydrochloride.

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay under subdued light or by using low-actinic glassware.]

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
Sample: Place 1 mL of Injection in a 125-mL separator, and add 1 N sodium hydroxide dropwise until the solution becomes opaque white. Extract with 50 mL of ether, wash the ether extract with 25 mL of water, and discard the washing. Filter the ether extract through a layer of anhydrous sodium sulfate into a beaker, and evaporate the filtrate by means of a stream of nitrogen to complete dryness. Dry at 100° for 3 h.
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
Solution A: Transfer 23.5 mL of 85% phosphoric acid into a 100-mL volumetric flask containing water, and dilute with water to volume.
Mobile phase: Add 20 mL of *Solution A* to 450 mL of water. To this solution add 5 mL of triethylamine, and adjust with 1 N sodium hydroxide to a pH of 3.0. Add 500 mL of acetonitrile, and dilute with water to 1000 mL. Filter, and degas.
System suitability solution: 2.0 mg/mL of benzyl alcohol, using appropriate amounts of 1% benzyl alcohol, and 0.1 mg/mL of USP Methotrimeprazine RS in *Mobile phase*
Standard solution: 0.1 mg/mL of USP Methotrimeprazine RS in *Mobile phase*
Sample solution: Nominally equivalent to 0.1 mg/mL of methotrimeprazine in *Mobile phase* from an appropriate amount of Injection

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between benzyl alcohol and methotrimeprazine, *System suitability solution*

Tailing factor: NMT 1.2, *System suitability solution*

Relative standard deviation: NMT 1.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methotrimeprazine ($C_{19}H_{24}N_2OS$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methotrimeprazine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methotrimeprazine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

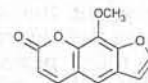
SPECIFIC TESTS

- **pH (791):** 3.0–5.0
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 17.9 USP Endotoxin Units/mg of methotrimeprazine
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.
- **USP REFERENCE STANDARDS (11)**
 USP Endotoxin RS
 USP Methotrimeprazine RS

Methoxsalen



$C_{12}H_8O_4$ 216.19

7H-Furo[3,2-g][1]benzopyran-7-one, 9-methoxy-

9-Methoxy-7H-furo[3,2-g][1]benzopyran-7-one [298-81-7].

» Methoxsalen contains not less than 98.0 percent and not more than 102.0 percent of $C_{12}H_8O_4$, calculated on the anhydrous basis.
Caution—Avoid contact with the skin.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards (11)—

USP Methoxsalen RS

Identification, Infrared Absorption (197K).

Melting range, Class I (741): between 143° and 148° .

Water Determination, Method I (921): not more than 0.5%.

Residue on ignition (281): not more than 0.1%, a 1-g specimen being used.

Delete the following:

• **Heavy metals, Method II** (231): 0.002%. (Official 1-Jan-2018)

Chromatographic impurities—Prepare a solution of it in chloroform containing about 20 mg per mL (*Solution A*). Dilute 1.0 mL of it with chloroform to 100.0 mL (*Solution B*). Apply 5- μ L portions of both solutions at points along a line about 2.5 cm from one edge of a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture and previously dried at 105° for 30 minutes. Develop the plate in a suitable chamber, without previous equilibration, using a mixture of 9 volumes of benzene and 1 volume of ethyl acetate, until the solvent front has moved to about 15 cm above the line of application. Remove the plate from the chamber, air-dry, and observe under long-wavelength UV light: any spot in the chromatogram from *Solution A*, other than the principal spot, is not more intense than the spot from *Solution B* (1.0%).

Assay—

Mobile phase—Prepare a solution of acetonitrile in water (35 in 100). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard preparation—Dissolve trioxsalen in alcohol to obtain a solution containing about 0.2 mg per mL.

Standard preparation—Using an accurately weighed quantity of USP Methoxsalen RS, prepare a solution in alcohol having a known concentration of about 0.2 mg per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 2.0 mL of *Internal standard preparation*, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 4 μ g of USP Methoxsalen RS per mL. Pass through a 0.45- μ m disk before using.

Assay preparation—Using 20 mg of Methoxsalen, accurately weighed, proceed as directed for *Standard preparation*.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm \times 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the analyte and internal standard peaks is not less than 4.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 2.1 for trioxsalen and 1.0 for methoxsalen. Calculate the quantity, in mg, of $C_{12}H_8O_4$, in the portion of Methoxsalen taken by the formula:

$$5C(R_U / R_S)$$

in which *C* is the concentration, in μ g per mL, of USP Methoxsalen RS in the *Standard preparation*; and *R_U* and *R_S* are the ratios of the peak responses of methoxsalen to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methoxsalen Capsules

» Methoxsalen Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methoxsalen ($C_{12}H_8O_4$).

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—Label the Capsules to state that Methoxsalen Hard Gelatin Capsules may not be interchangeable with Methoxsalen Soft Gelatin Capsules without retitration of the patient.

USP Reference standards (11)—

USP Methoxsalen RS

Identification—

A: The retention time exhibited by methoxsalen in the chromatogram of the *Assay preparation* corresponds to that of methoxsalen in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

B: Place one Capsule in 50 mL of alcohol contained in a high-speed glass blender jar and blend thoroughly until the shell is completely dispersed. Dilute a portion quantitatively with alcohol to obtain a solution having a concentration of about 4 μ g per mL: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Methoxsalen RS, concomitantly measured.

Dissolution (711)—

FOR SOFT GELATIN CAPSULES—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{12}H_8O_4$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 300 nm using filtered portions of the solution under test, suitably diluted with water, if necessary, in comparison with a *Standard solution* having a known concentration of USP Methoxsalen RS in the same *Medium*. [NOTE—An amount of alcohol not to exceed 1% of the total volume of the *Standard solution* may be used to bring the *Reference Standard* into solution prior to dilution with *Medium*.]

Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{12}H_8O_4$ is dissolved in 45 minutes.

FOR HARD GELATIN CAPSULES—

Medium: water; 900 mL.

Apparatus 1: 150 rpm.

Time: 90 minutes.

Procedure—Determine the amount of $C_{12}H_8O_4$ dissolved from UV absorbances at the wavelength of maximum absorbances at about 252 nm of filtered portions of the solution under test in comparison with a *Standard solution* having a known concentration of USP Methoxsalen RS prepared in alcohol and diluted with water.

Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{12}H_8O_4$ is dissolved in 90 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Prepare a solution in alcohol having an accurately known concentration of 0.2 mg of USP Methoxsalen RS per mL. Pipet 2.0 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation—

FOR HARD GELATIN CAPSULES—Place not less than 10 Capsules in a high-speed glass blender jar containing 100.0 mL of alcohol, and blend thoroughly. Transfer an accurately measured volume of the aliquot from the blender jar, equivalent to about 2 mg of Methoxsalen, to a 50-mL volumetric flask, dilute with alcohol to volume, mix, and filter. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and filter.

FOR SOFT GELATIN CAPSULES—Place the end of a long-stem glass funnel on a 250-mL volumetric flask, punch a hole at each end of a Capsule with a syringe containing 15 mL of alcohol, and rinse the contents into the flask. Cut the Capsule shell with a scalpel, and wash the inside of the shell with 15 mL of alcohol into the same flask. Repeat these steps for not less than 4 additional Capsules, and collect the rinse. Wash the funnel, and collect the rinse in the same flask. Dilute with alcohol to volume, and mix. Transfer an accurately measured volume of this solution, equivalent to about 2 mg of methoxsalen, to a 50-mL volumetric flask, dilute with alcohol to volume, mix, and filter. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and filter.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in percentage of the label claim, of methoxsalen (C₁₂H₈O₄) in the portion of the Capsule taken by the formula:

$$100(C_s / C_u)(r_u / r_s)$$

in which C_s is the concentration, in mg per mL, of USP Methoxsalen RS in the *Standard preparation*; C_u is the nominal concentration, in mg per mL, of methoxsalen in the *Assay preparation*, based on the label claim; and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methoxsalen Topical Solution

» Methoxsalen Topical Solution is a solution of Methoxsalen in a suitable vehicle. It contains not less than 9.2 mg and not more than 10.8 mg of methoxsalen (C₁₂H₈O₄) per mL.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Methoxsalen RS

Identification—Transfer a volume of Topical Solution to a 100-mL volumetric flask, and dilute quantitatively and stepwise with alcohol to obtain a concentration of about 8 µg per mL: the UV absorption spectrum of this solution exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Methoxsalen RS, concomitantly measured.

Alcohol Determination (611) (if present): between 66.5% and 77.0% of C₂H₅OH.

Assay—

*Mobile phase, Internal standard preparation, Standard preparation, and Chromatographic system—*Prepare as directed in the Assay under *Methoxsalen*.

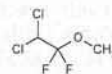
Assay preparation—Transfer an accurately measured volume of Topical Solution, equivalent to about 20 mg of methoxsalen, to a 100-mL volumetric flask. Dilute with alcohol to volume, and mix. Transfer 2.0 mL of this solution and 2.0 mL of *Internal standard preparation* to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Pass through a 0.45-µm disk before using.

Procedure—Proceed as directed for *Procedure* in the Assay under *Methoxsalen*. Calculate the quantity, in mg, of methoxsalen (C₁₂H₈O₄) in each mL of the Topical Solution taken by the formula:

$$5(C/V)(R_u / R_s)$$

in which V is the volume, in mL, of Topical Solution taken, and the other terms are as defined therein.

Methoxyflurane



C₃H₄Cl₂F₂O

164.97

Ethane, 2,2-dichloro-1,1-difluoro-1-methoxy-;
2,2-Dichloro-1,1-difluoroethyl methyl ether [76-38-0].

DEFINITION

Methoxyflurane contains NLT 99.9% and NMT 100.0% of methoxyflurane (C₃H₄Cl₂F₂O). It may contain a suitable stabilizer.

IDENTIFICATION

- A. INFRARED ABSORPTION**

Standard solution: USP Methoxyflurane RS (1 in 20) in chloroform

Sample solution: Methoxyflurane (1 in 20) in chloroform

Acceptance criteria: The IR absorption spectrum of the *Sample solution* exhibits maxima only at the same wavelengths as those of the *Standard solution*.

- B.**

Sample: Methoxyflurane

Analysis: To 1 mL of the *Sample* in a test tube add 1 mL of sulfuric acid.

Acceptance criteria: The *Sample* forms a layer over the acid (distinction from halothane).

- C.**

Analysis: Cautiously heat the contents of the test tube from *Identification* test B with agitation.

Acceptance criteria: The interface disappears, and hydrofluoric acid is evolved (distinction from chloroform, trichloroethylene, and halothane).

ASSAY

- PROCEDURE**

Sample: Methoxyflurane

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Thermal conductivity

Column: 4-mm × 3-m; stainless steel packed with liquid phase G11 on support S1A

Temperatures

Injection port: 150°

Column: 100°–110°

Carrier gas: Dry helium

Flow rate: 60 mL/min

Injection volume: NMT 30 µL

AnalysisSample: *Sample*Calculate the percentage of methoxyflurane (C₃H₄Cl₂F₂O) in the portion of Methoxyflurane taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 r_U = peak area of methoxyflurane r_T = sum of all the peak areas

Acceptance criteria: 99.9%–100.0%

SPECIFIC TESTS

- **SPECIFIC GRAVITY** (841): 1.420–1.425

- **ACIDITY**

Sample: 25 mL of Methoxyflurane

Titrimetric system

Mode: Direct titration

Titrant: 0.010 N sodium hydroxide

Endpoint detection: Visual

Analysis: Shake the *Sample* with 25 mL of carbon dioxide-free water for 2 min, and allow the layers to separate. Add 1 drop of methyl red TS to the water extract, boil for 1 min, and titrate with *Titrant*.

Acceptance criteria: NMT 0.50 mL of *Titrant* is required to produce a distinct yellow color.

- **WATER DETERMINATION, Method I** (921): NMT 0.1%

- **LIMIT OF NONVOLATILE RESIDUE**

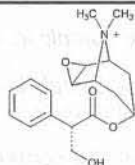
Sample: 50 mL of Methoxyflurane

Analysis: Evaporate the *Sample* at room temperature in a tared evaporating dish, and dry the residue at 105° for 1 h.

Acceptance criteria: The weight of the residue does not exceed 1 mg.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** (11)
USP Methoxyflurane RS

Methscopolamine BromideC₁₈H₂₄BrNO₄ 398.29

3-Oxa-9-azoniatricyclo[3.3.1.0^{2,4}]nonane, 7-(3-hydroxy-1-oxo-2-phenylpropoxy)-9,9-dimethyl-, bromide, [7(S)-(1α,2β,4β,5α,7β)-

6β,7β-Epoxy-3α-hydroxy-8-methyl-1αH,5αH-tropanium bromide (-)-tropate [155-41-9].

» Methscopolamine Bromide contains not less than 97.0 percent and not more than 103.0 percent of C₁₈H₂₄BrNO₄, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store at room temperature.

USP Reference standards (11)—

USP Methscopolamine Bromide RS

USP Scopolamine Hydrobromide RS

IdentificationA: *Infrared Absorption* (197K).

B: A solution (1 in 20) meets the requirements of the tests for *Bromide* (191).

Specific rotation (781): between –21° and –25°, determined in a solution containing 500 mg in each 10 mL.

Loss on drying (731)—Dry it at 105° for 2 hours: it loses not more than 2.0% of its weight.

Residue on ignition (281): not more than 0.1%.

Chromatographic purity

Buffer solution, Solution A, Solution B, and Mobile phase—Proceed as directed in the *Assay*.

Standard solution—Prepare as directed for the *Standard preparation* in the *Assay*.

Diluted standard solution—Dilute 5 µL of the *Standard solution* with *Solution A* to 10.0 mL.

Test solution—Prepare as directed for the *Assay preparation*.

Scopolamine hydrobromide solution—Dissolve an accurately weighed quantity of USP Scopolamine Hydrobromide RS in *Solution A* to obtain a solution having a known concentration of about 0.05 mg per mL.

System suitability solution—Dissolve about 50 mg of USP Methscopolamine Bromide RS in *Solution A*, add 1.0 mL of *Scopolamine hydrobromide solution*, and dilute with *Solution A* to 50.0 mL. This solution contains about 0.1% of scopolamine hydrobromide.

Chromatographic system (see *Chromatography* (621))—Proceed as directed in the *Assay*. In addition, chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, R , between methscopolamine and scopolamine is not less than 1.5; and the tailing factor for the methscopolamine peak is not more than 2.0.

Procedure—Separately inject equal volumes (about 5 µL) of the *Diluted standard solution* and the *Test solution* into the chromatograph, record the chromatogram for four times the retention time of methscopolamine, and measure the responses for the major peaks. Disregard any peak with an area less than that of the methscopolamine peak in the chromatogram obtained from the *Diluted standard solution*, and disregard any peak that is due to *Solution A*. Calculate the percentage of each impurity in the portion of Methscopolamine Bromide taken by the formula:

$$100F(r_i/r_s)$$

in which F is the relative response factor for the methscopolamine bromide impurities (see *Table 1*); r_i is the peak area of any impurity obtained from the *Test solution*; and r_s is the peak area of methscopolamine obtained from the chromatogram of the *Test solution*: not more than 0.1% of any individual impurity is found; and not more than 0.5% of total impurities is found.

Table 1

Name	Relative Retention Time	Relative Response Factor (F)
Tropic acid	0.4	0.4
Scopolamine hydrobromide	0.9	1.0
Methylatropine bromide	1.2	1.0
Apomethscopolamine bromide	3.5	0.6
Any other impurity	—	1.0

Assay—

Buffer solution—Prepare a solution containing 5.16 g of sodium 1-hexanesulfonate monohydrate and 3.40 g of monobasic potassium phosphate in 1000 mL of water, adjust with 1 M phosphoric acid to a pH of 2.8, and mix.

Solution A—Mix 850 mL of *Buffer solution* and 150 mL of acetonitrile, filter, and degas.

Solution B—Mix 500 mL of *Buffer solution* and 500 mL of acetonitrile, filter, and degas.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Methscopolamine Bromide RS in *Solution A* to obtain a solution having a known concentration of about 1.0 mg per mL.

Assay preparation—Transfer about 50 mg of Methscopolamine Bromide, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Solution A* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 10-cm column that contains packing L1. The flow rate is about 3 mL per minute. The column temperature is maintained at 50°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–3	100	0	isocratic
3–10	100→85	0→15	linear gradient
10–10.1	85→100	15→0	linear gradient
10.1–13	100	0	re-equilibration

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for six replicate injections is not greater than 1%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak area responses. Calculate the quantity, in mg, of $C_{18}H_{24}BrNO_4$ in the portion of Methscopolamine Bromide taken by the formula:

$$50C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Methscopolamine Bromide RS in the *Standard preparation*; and r_U and r_S are the peak area responses of methscopolamine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methscopolamine Bromide Tablets

» Methscopolamine Bromide Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of methscopolamine bromide ($C_{18}H_{24}BrNO_4$).

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.

USP Reference standards (11)—
USP Methscopolamine Bromide RS

Identification—

A: Thin-Layer Chromatographic Identification Test (201)—

pH 7.3 Dye-buffer solution—Prepare a solution containing, in each 500 mL, 200 mg of bromothymol blue, 3.2 mL of 0.1 N sodium hydroxide, 577.5 mg of citric acid monohydrate, and 6.3 mg of anhydrous dibasic sodium phosphate.

Test solution—Finely powder 1 Tablet, and transfer an amount, equivalent to about 0.5 mg of methscopolamine bromide, to a suitable container. Add 20 mL of water, heat for 5 minutes on a steam bath with frequent agitation, and centrifuge to obtain a clear supernatant. Transfer 10 mL of the supernatant to a vessel containing 10 mL of chloroform and 10 mL of pH 7.3 Dye-buffer solution. Shake vigorously for 3 minutes, centrifuge, and transfer 8 mL of the chloroform layer to a suitable container. Evaporate to dryness, and dissolve the residue in 1 mL of chloroform.

Standard solution—Prepare a solution in water containing about 0.025 mg of USP Methscopolamine Bromide RS per mL, and treat as directed above, beginning with "Transfer 10 mL of the supernatant."

Application volume: 50 µL.

Developing solvent system—In a suitable container, mix water, butyl alcohol, and glacial acetic acid (5:4:1), then transfer a measured volume of the upper organic layer to a suitable container, and mix with a volume of alcohol equivalent to 20% of the volume of the organic layer.

Procedure—Allow the solvent front to move about three-fourths of the length of the plate, remove the plate from the developing chamber, mark the solvent front, and dry the plate under a current of air for 30 minutes. Spray the plate evenly with potassium-bismuth iodide TS: the chromatogram of the *Test solution* shows a bright orange spot on a yellow background corresponding in R_f value (about 0.25) to that in the chromatogram obtained from the *Standard solution*. [NOTE—Bromothymol blue produces a dark yellow spot at an R_f value of about 0.8.]

B: Powder a number of Tablets, equivalent to about 5 mg of methscopolamine bromide, digest with 5 mL of water for 10 minutes, and filter: a portion of the clear solution so obtained responds to the test for *Bromide* (191).

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 500 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the percentage of the labeled amount of methscopolamine bromide dissolved using the following method.

pH 3.0 Phosphate buffer—Dissolve 5.44 g of monobasic potassium phosphate in 1 L of water. Adjust with 1 N phosphoric acid to a pH of 3.0.

Mobile phase—Prepare a filtered and degassed mixture of pH 3.0 Phosphate buffer and methanol (3:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard solution—Dissolve an accurately weighed quantity of USP Methscopolamine Bromide RS in *Medium*, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration similar to the one expected in the *Test solution*.

Test solution—Use portions of the solution under test that have been passed through a 0.45-µm PTFE filter.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 204-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 30°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of methscopolamine bromide dissolved by the formula:

$$\frac{r_U \times C_S \times 500 \times 100}{r_S \times LC}$$

in which r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively; C_S is the concentration, in mg per mL, of USP Methscopolamine Bromide RS in the *Standard solution*; 500 is the volume, in mL, of *Medium*; 100 is the factor for conversion to percentage; and LC is the tablet label claim, in mg.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{18}H_{24}BrNO_4$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase—Prepare a solution containing 2.6 g of decyl sodium sulfate in 450 mL of water. Add 550 mL of methanol, adjust with 1 N sulfuric acid to a pH of 3.5, mix, filter, and degas.

Standard preparation—Transfer about 25 mg of USP Methscopolamine Bromide RS, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Assay preparation—

FOR TABLETS THAT CONTAIN 2.5 MG OF METHSCOPOLAMINE BROMIDE—Place 10 Tablets in a 100-mL volumetric flask, add about 50 mL of *Mobile phase*, and sonicate for 30 minutes. Shake by mechanical means for 30 minutes, dilute with *Mobile phase* to volume, and mix. Pass a portion through a 0.45- μ m PTFE filter, discarding the first 2 to 3 mL of the filtrate.

FOR TABLETS THAT CONTAIN 5 MG OF METHSCOPOLAMINE BROMIDE—Place 10 Tablets in a 200-mL volumetric flask, add about 100 mL of *Mobile phase*, and sonicate for 30 minutes. Shake by mechanical means for 30 minutes, dilute with *Mobile phase* to volume, and mix. Pass a portion through a 0.45- μ m PTFE filter, discarding the first 2 to 3 mL of the filtrate.

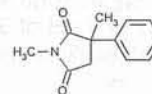
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm \times 25-cm column that contains packing L1. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject a volume (about 50 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the quantity, in mg, of methscopolamine bromide ($C_{18}H_{24}BrNO_4$) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Methscopolamine Bromide RS in the *Standard preparation*; and r_U and r_S are the peak responses of the methscopolamine bromide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methsuximide



$C_{12}H_{13}NO_2$

203.24

2,5-Pyrrolidinedione, 1,3-dimethyl-3-phenyl-, (\pm)-; (\pm)-N,2-Dimethyl-2-phenylsuccinimide [77-41-8].

DEFINITION

Methsuximide contains NLT 97.0% and NMT 103.0% of methsuximide ($C_{12}H_{13}NO_2$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile and water (9:11)

Standard solution: 0.6 mg/mL of USP Methsuximide RS in *Mobile phase*

Sample solution: 0.6 mg/mL of Methsuximide in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 5800 theoretical plates

Tailing factor: NMT 1.3

Relative standard deviation: NMT 0.6%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methsuximide ($C_{12}H_{13}NO_2$) in the portion of Methsuximide taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methsuximide RS in the *Standard solution* (mg/mL)

C_U = concentration of Methsuximide in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

• ORGANIC IMPURITIES

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 0.6 mg/mL of Methsuximide in *Mobile phase*

Standard solution: 0.006 mg/mL of USP Methsuximide RS in *Mobile phase*

Sample solution: 6.0 mg/mL of Methsuximide in *Mobile phase*

System suitabilitySample: *System suitability solution***Suitability requirements**

Column efficiency: NLT 5800 theoretical plates

Tailing factor: NMT 1.3

Relative standard deviation: NMT 0.6%

AnalysisSamples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Methsuximide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response for each impurity from the *Sample solution* r_S = peak response for methsuximide from the *Standard solution* C_S = concentration of USP Methsuximide RS in the *Standard solution* (mg/mL) C_U = concentration of Methsuximide in the *Sample solution* (mg/mL)**Acceptance criteria**

Any individual impurity: NMT 0.1%

Total impurities: NMT 2.0%

SPECIFIC TESTS• **LOSS ON DRYING (731)**

Analysis: Dry a sample over phosphorus pentoxide for 16 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in tight containers.• **USP REFERENCE STANDARDS (11)**

USP Methsuximide RS

Methsuximide Capsules

DEFINITIONMethsuximide Capsules contain NLT 92.0% and NMT 108.0% of the labeled amount of methsuximide ($C_{12}H_{13}NO_2$).**IDENTIFICATION**• **A. INFRARED ABSORPTION (197K)**

Sample: Mix a portion of the content of Capsules, equivalent to 200 mg of methsuximide with 25 mL of water in a separator, extract with 50 mL of ether, and discard the aqueous layer. Wash the ether extract with 25 mL of water, and discard the water. Filter the extract, evaporate with the aid of a current of warm air to dryness, and dry the methsuximide over phosphorus pentoxide for 16 h.

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.**ASSAY**• **PROCEDURE****Mobile phase:** Acetonitrile and water (45:55). Filter, and degas.**Standard solution:** 0.6 mg/mL of USP Methsuximide RS in *Mobile phase***Sample stock solution:** Place 10 Capsules in a 500-mL volumetric flask, and add 280 mL of water. Sonicate in a water bath at 40°–50°, with occasional shaking, until the Capsules have broken, and cool to room temperature. Dilute with acetonitrile to volume, mix, and filter.**Sample solution:** Nominally 0.6 mg/mL of methsuximide prepared from *Sample stock solution* in *Mobile phase***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitabilitySample: *Standard solution***Suitability requirements**

Column efficiency: NLT 2100 theoretical plates

Relative standard deviation: NMT 1.5%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of methsuximide ($C_{12}H_{13}NO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of methsuximide from the *Sample solution* r_S = peak response of methsuximide from the *Standard solution* C_S = concentration of USP Methsuximide RS in the *Standard solution* (mg/mL) C_U = nominal concentration of methsuximide in the *Sample solution* (mg/mL)

Acceptance criteria: 92.0%–108.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: Water; 900 mL

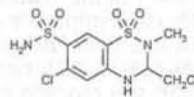
Apparatus 1: 100 rpm

Time: 120 min

Analysis: Determine the percentage of the labeled amount of methsuximide ($C_{12}H_{13}NO_2$) dissolved by using the procedure set forth in the Assay, making any necessary adjustments.Tolerances: NLT 75% (Q) of the labeled amount of methsuximide ($C_{12}H_{13}NO_2$) is dissolved.• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid exposure to excessive heat.• **USP REFERENCE STANDARDS (11)**

USP Methsuximide RS

Methyclothiazide

 $C_9H_{11}Cl_2N_3O_4S_2$ 360.242*H*-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-3-(chloromethyl)-3,4-dihydro-2-methyl-, 1,1-dioxide, (±)-, (±)-6-Chloro-3-(chloromethyl)-3,4-dihydro-2-methyl-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [135-07-9].» Methyclothiazide contains not less than 97.0 percent and not more than 102.0 percent of $C_9H_{11}Cl_2N_3O_4S_2$, calculated on the dried basis.**Packaging and storage**—Preserve in well-closed containers.

USP Reference standards (11)—

USP Methyclothiazide RS

USP Methyclothiazide Related Compound A RS

4-Amino-6-chloro-*N*³-methyl-*m*-benzenedisulfonamide.
 $C_7H_{10}ClN_3O_4S_2$ 299.76**Identification—****A:** Infrared Absorption (197K).**B:** Ultraviolet Absorption (197U)—*Solution:* 20 µg per mL.*Medium:* methanol.**C:** Fuse about 100 mg of it with a pellet of sodium hydroxide: the ammonia fumes produced turn moistened red litmus paper blue. The fused mixture responds to the test for *Sulfite* (191).**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.**Residue on ignition** (281): not more than 0.2%.**Chloride** (221)—Shake 750 mg with 25 mL of water for 2 minutes, filter through a suitable membrane filter, and add 4 or 5 drops of 2 N nitric acid: the acidified filtrate shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.02%).**Selenium** (291): 0.003%.**Delete the following:****•Heavy metals, Method II** (231): 0.002%. • (Official 1-Jan-2018)**Diazotizable substances—****Standard preparation**—Transfer about 10 mg of USP Methyclothiazide Related Compound A RS, accurately weighed, to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix. Pipet 25 mL of the solution into a 100-mL volumetric flask, dilute with acetonitrile to volume, and mix. Each mL of *Standard preparation* contains about 50 µg of the Reference Standard.**Test preparation**—Transfer about 500 mg of Methyclothiazide, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix.**Procedure**—Pipet 2 mL each of the *Standard preparation* and the *Test preparation* into separate 50-mL volumetric flasks. Pipet 2 mL of acetonitrile into a third 50-mL flask to provide the blank. To each flask add 4 mL of 0.1 N hydrochloric acid, and mix. Add 3.0 mL of sodium nitrite solution (1 in 200) to each flask, mix, and place the flasks in an ice bath for 5 minutes, shaking occasionally. Add to each flask 3.0 mL of ammonium sulfamate solution (1 in 50), mix, and allow the flasks to remain in the ice bath for 1 additional minute. Remove the flasks from the ice bath, add 1.0 mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000), and mix. Allow the flasks to stand at room temperature for 1 minute, then dilute with water to volume, and mix. Concomitantly determine the absorbances of the solutions obtained from the *Standard preparation* and the *Test preparation* in 1-cm cells at 525 nm, with a suitable spectrophotometer, using the reagent blank to set the instrument. The absorbance of the solution from the *Test preparation* does not exceed that of the solution from the *Standard preparation*, corresponding to not more than 1.0% of diazotizable substances.**Assay**—Transfer about 350 mg of Methyclothiazide, accurately weighed, to a 250-mL conical flask, add 40 mL of a 1 in 20 solution of potassium hydroxide in methanol, and reflux at full boil for 1 hour. Cool, rinse the inner walls of the condenser with 20 mL of water and two 20-mL portions of methanol, add 10 mL of glacial acetic acid and 2 drops of eosin Y TS, and titrate with 0.1 N silver nitrate VS to the first appearance of a definite pink color. Each mL of 0.1 N silver nitrate is equivalent to 36.02 mg of $C_7H_{11}Cl_2N_3O_4S_2$.**Methyclothiazide Tablets**» Methyclothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methyclothiazide ($C_9H_{11}Cl_2N_3O_4S_2$).**Packaging and storage**—Preserve in well-closed containers.**USP Reference standards (11)—**

USP Methyclothiazide RS

Identification, Ultraviolet Absorption (197U)—**Solution**—Powder a number of Tablets, equivalent to about 50 mg of methyclothiazide, and transfer to a 100-mL volumetric flask with the aid of methanol. Add about 60 mL of methanol, and shake the flask for 1 hour. Dilute with methanol to volume, mix, and centrifuge a portion of the solution. Pipet 2 mL of the clear supernatant into a second 100-mL volumetric flask, dilute with methanol to volume, and mix: the UV absorption spectrum of this solution exhibits maxima and minima only at the same wavelengths as that of a similar solution of USP Methyclothiazide RS.**Dissolution** (711)—*Medium:* 0.01 N hydrochloric acid; 900 mL.*Apparatus 2:* 50 rpm.*Time:* 60 minutes.**Procedure**—Determine the amount of $C_9H_{11}Cl_2N_3O_4S_2$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 270 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Methyclothiazide RS in the same *Medium*. An amount of alcohol not to exceed 1% of the total volume of the Standard solution may be used to dissolve USP Methyclothiazide RS prior to dilution with *Dissolution Medium*.**Tolerances**—Not less than 70% (*Q*) of the labeled amount of $C_9H_{11}Cl_2N_3O_4S_2$ is dissolved in 60 minutes.**Uniformity of dosage units** (905): meet the requirements.**Procedure for content uniformity**—Transfer 1 finely powdered Tablet to a 50-mL volumetric flask, add about 30 mL of methanol, and shake by mechanical means for 1 hour. Dilute with methanol to volume, mix, and centrifuge a portion of the mixture. Dilute quantitatively with methanol to obtain a solution containing approximately 10 µg per mL of methyclothiazide. Concomitantly determine the absorbances of this solution and a Standard solution of USP Methyclothiazide RS in the same medium, having a known concentration of about 10 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 267 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_9H_{11}Cl_2N_3O_4S_2$ in the Tablet taken by the formula:

$$(TC / D)(A_U / A_S)$$

in which *T* is the labeled quantity, in mg, of methyclothiazide in the Tablet; *C* is the concentration, in µg per mL, of USP Methyclothiazide RS in the Standard solution; *D* is the concentration, in µg per mL, of methyclothiazide in the solution from the Tablet, based upon the labeled quantity per Tablet and the extent of dilution; and *A_U* and *A_S* are the absorbances of the solution from the Tablet and the Standard solution, respectively.**Assay—****Standard preparation**—Transfer about 20 mg of USP Methyclothiazide RS, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer

10.0 mL of this solution to a 200-mL volumetric flask, add chloroform to volume, and mix.

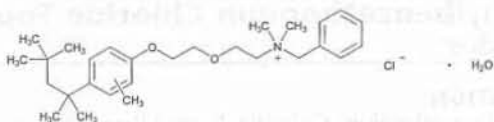
Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2 mg of methyclothiazide, to a 150-mL beaker, add 2.0 mL of methanol, mix, allow the mixture to stand for 30 minutes while taking precautions against loss of solvent, add 2.0 mL of 0.1 M sodium bicarbonate, and mix.

Procedure—[NOTE—Use water-saturated solvents throughout this procedure.] Mix about 3 g of chromatographic siliceous earth with 2.0 mL of 0.1 M sodium bicarbonate in a 150-mL beaker. Pack the mixture into a 25- × 200-mm chromatographic column. Add 4 g of chromatographic siliceous earth to the *Assay preparation*, mix, transfer the mixture to the column, and pack. Dry-wash the beaker with 1 g of the siliceous earth mixed with 3 drops of water, and transfer to the column. Place a small pad of glass wool above the column packing, pass 75 mL of a mixture of isooctane and ether (9:1) through the column, and discard the eluate. Using a 200-mL volumetric flask as a receiver, pass 100 mL of chloroform through the column, wash the tip of column with ether, add 10.0 mL of methanol, dilute with chloroform to volume, and mix. Concomitantly determine the absorbances of this solution and the *Standard preparation* at the wavelength of maximum absorbance at about 268 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of methyclothiazide ($C_9H_{11}Cl_2N_3O_4S_2$) in the portion of Tablets taken by the formula:

$$0.2C(A_U / A_S)$$

in which C is the concentration, in μg per mL, of USP Methyclothiazide RS in the *Standard preparation*; and A_U and A_S are the absorbances of the solution from the *Assay preparation* and the *Standard preparation*, respectively.

Methylbenzethonium Chloride



$C_{28}H_{44}ClNO_2 \cdot H_2O$ 480.12

$C_{28}H_{44}ClNO_2$ 462.12

Benzenemethanaminium, *N,N*-dimethyl-*N*-[2-[2-[methyl-4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]-, chloride, monohydrate;

Benzyltrimethyl[2-[2-[[4-(1,1,3,3-tetramethylbutyl)tolyl]oxy]ethoxy]ethyl]ammonium chloride monohydrate [1320-44-1].

Anhydrous [25155-18-4].

DEFINITION

Methylbenzethonium Chloride contains NLT 97.0% and NMT 103.0% of $C_{28}H_{44}ClNO_2$, calculated on the dried basis.

IDENTIFICATION

A. PROCEDURE

Analysis: To 1 mL of solution (1 in 100) add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS.

Acceptance criteria: A white precipitate, which is insoluble in 2 N nitric acid and soluble in 6 N ammonium hydroxide, is formed.

B. INFRARED ABSORPTION (197K)

ASSAY

PROCEDURE

Buffer: Dilute 20 mL of triethylamine with water to 1000 mL, and adjust the pH to 3.0 with phosphoric acid.

Mobile phase: Acetonitrile and Buffer (45:55)

Standard solution: 0.15 mg/mL of USP

Methylbenzethonium Chloride RS in *Mobile phase*

System suitability solution: 0.15 mg/mL each of USP Benzethonium Chloride RS and USP

Methylbenzethonium Chloride RS in *Mobile phase*

Sample solution: 0.15 mg/mL of methylbenzethonium chloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 15-cm; 5- μm packing L7

Column temperature: 40°

Flow rate: 1 mL/min

Injection size: 10 μL

Run time: 1.5 times the retention time of the methylbenzethonium peak

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for benzethonium and methylbenzethonium are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5.0 between the benzethonium and methylbenzethonium peaks

Tailing factor: NMT 1.8 for the methylbenzethonium peak

Relative standard deviation: NMT 0.5% for the methylbenzethonium peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylbenzethonium chloride ($C_{28}H_{44}ClNO_2 \cdot H_2O$) in the portion of Methylbenzethonium Chloride taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

r_U = peak response of methylbenzethonium from the *Sample solution*

r_S = peak response of methylbenzethonium from the *Standard solution*

C_S = concentration of USP Methylbenzethonium Chloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylbenzethonium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** (741): 159°–163°, the specimen having been previously dried

• **LOSS ON DRYING** (731): Dry a sample at 105° for 4 h: it loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Benzethonium Chloride RS

USP Methylbenzethonium Chloride RS

Methylbenzethonium Chloride Lotion

DEFINITION

Methylbenzethonium Chloride Lotion is an emulsion containing NLT 90.0% and NMT 110.0% of the labeled amount of methylbenzethonium chloride ($C_{28}H_{44}ClNO_2 \cdot H_2O$).

IDENTIFICATION

- **A.**
Sample: Suspend 0.5 mL of methylbenzethonium chloride lotion in 20 mL of water.
Analysis: Add 0.1 g of sodium carbonate, 1 mL of bromophenol blue TS, and 10 mL of chloroform to the *Sample*, and shake the mixture.
Acceptance criteria: The chloroform layer is blue.

ASSAY

- **PROCEDURE**
Standard stock solution: 4.446 mg/mL of USP Docusate Sodium RS in isopropyl alcohol (equivalent to 0.01 M of USP Docusate Sodium RS). Store this solution in a tightly-stoppered glass container. Prepare the *Standard solution* on the day of use.
Standard solution 44.46 µg/mL of USP Docusate Sodium RS in water from the *Standard stock solution* (equivalent to 0.1 mM of USP Docusate Sodium RS)
Sample solution: Transfer an amount of Lotion, equivalent to 0.5 mg of methylbenzethonium chloride, to a glass-stoppered, 50-mL cylinder. Add 5 mL of chloroform (freshly purified by shaking 100 mL with 10 g of silica gel, allowing to settle, and withdrawing the supernatant), 5 mL of phosphoric acid solution (1 in 10), and 1 mL of 0.05 mg/mL of safranin O solution.
Analysis: Titrate the *Sample solution* with the *Standard solution* until 1 mL from the endpoint, then shake the stoppered tube vigorously for about 2 min, and continue the titration in 0.1-mL increments, shaking vigorously after each addition, until a pink color appears in the chloroform layer. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of *Standard solution* is equivalent to 48.01 µg of methylbenzethonium chloride ($C_{28}H_{44}ClNO_2 \cdot H_2O$).
Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH (791):** 5.2–6.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
 USP Docusate Sodium RS

Methylbenzethonium Chloride Ointment

DEFINITION

Methylbenzethonium Chloride Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of methylbenzethonium chloride ($C_{28}H_{44}ClNO_2 \cdot H_2O$).

IDENTIFICATION

- **A.**
Sample: Suspend 0.5 g of methylbenzethonium chloride ointment in 10 mL of water.
Analysis: Add 0.1 g of sodium carbonate, 1 mL of bromophenol blue TS, and 10 mL of chloroform to the *Sample*, and shake the mixture.

Acceptance criteria: The chloroform layer is blue.

ASSAY

- **PROCEDURE**
Standard stock solution: 4.446 mg/mL of USP Docusate Sodium RS in isopropyl alcohol (equivalent to 0.01 M of USP Docusate Sodium RS). Store this solution in a tightly-stoppered glass container. Prepare the *Standard solution* on the day of use.
Standard solution 44.46 µg/mL of USP Docusate Sodium RS in water from the *Standard stock solution* (equivalent to 0.1 mM of USP Docusate Sodium RS)
Sample solution: Transfer an amount of Ointment, equivalent to 0.5 mg of methylbenzethonium chloride, to a glass-stoppered, 50-mL cylinder. Add 5 mL of chloroform (freshly purified by shaking 100 mL with 10 g of silica gel, allowing to settle, and withdrawing the supernatant), 5 mL of phosphoric acid solution (1 in 10), and 1 mL of 0.05 mg/mL of safranin O solution.
Analysis: Titrate the *Sample solution* with the *Standard solution* until 1 mL from the endpoint, then shake the stoppered tube vigorously for about 2 min, and continue the titration in 0.1-mL increments, shaking vigorously after each addition, until a pink color appears in the chloroform layer. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of *Standard solution* is equivalent to 48.01 µg of methylbenzethonium chloride ($C_{28}H_{44}ClNO_2 \cdot H_2O$).
Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH (791):** 5.0–7.0, in a dispersion of 10 mg/mL of the sample in carbon dioxide-free water

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers.
- **USP REFERENCE STANDARDS (11)**
 USP Docusate Sodium RS

Methylbenzethonium Chloride Topical Powder

DEFINITION

Methylbenzethonium Chloride Topical Powder contains NLT 85.0% and NMT 115.0% of the labeled amount of methylbenzethonium chloride ($C_{28}H_{44}ClNO_2 \cdot H_2O$) in a suitable fine powder base, free from grittiness.

IDENTIFICATION

- **A.**
Sample: Suspend 0.1 g of methylbenzethonium chloride topical powder in 10 mL of water.
Analysis: Add 0.1 g of sodium carbonate, 1 mL of bromophenol blue TS, and 10 mL of chloroform to the *Sample*, and shake the mixture.
Acceptance criteria: The chloroform layer is blue.

ASSAY

- **PROCEDURE**
Standard stock solution: 4.446 mg/mL of USP Docusate Sodium RS in isopropyl alcohol (equivalent to 0.01 M of USP Docusate Sodium RS). Store this solution in a tightly-stoppered glass container. Prepare the *Standard solution* on the day of use.
Standard solution: 44.46 µg/mL of USP Docusate Sodium RS in water from the *Standard stock solution* (equivalent to 0.1 mM of USP Docusate Sodium RS)
Sample solution: Transfer an amount of Topical Powder, equivalent to 0.5 mg of methylbenzethonium chloride, to a glass-stoppered, 50-mL cylinder. Add 5 mL of

chloroform (freshly purified by shaking 100 mL with 10 g of silica gel, allowing to settle, and withdrawing the supernatant), 5 mL of phosphoric acid solution (1 in 10), and 1 mL of 0.05 mg/mL of safranin O solution.

Analysis: Titrate the *Sample solution* with the *Standard solution* until 1 mL from the endpoint, then shake the stoppered tube vigorously for about 2 min, and continue the titration in 0.1-mL increments, shaking vigorously after each addition, until a pink color appears in the chloroform layer. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of *Standard solution* is equivalent to 48.01 µg of methylbenzethonium chloride ($C_{28}H_{44}ClNO_2 \cdot H_2O$).

Acceptance criteria: 85.0%–115.0%

SPECIFIC TESTS

- **pH (791):** 9.0–10.5, in a dispersion of 10 mg/mL of the sample in carbon dioxide-free water
- **POWDER FINENESS (811):** NLT 99% of it passes through a No. 200 sieve.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
USP Docusate Sodium RS

Methylcellulose

Portions of the monograph text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (•) to specify this fact.

Cellulose, methyl ether;

Cellulose methyl ether [9004-67-5].

DEFINITION

Methylcellulose is a methyl ether of cellulose. When dried at 105° for 1 h, it contains NLT 26.0% and NMT 33.0% of methoxy ($-OCH_3$) groups.

IDENTIFICATION

- **A.**
Sample: 1 g
Analysis: Evenly distribute the *Sample* onto the surface of 100 mL of water in a beaker, tapping the top of the beaker gently, if necessary, to ensure a uniform layer on the surface, and allow to stand for 1–2 min.
Acceptance criteria: The powdered material aggregates on the surface.
- **B.**
Sample: 1 g
Analysis: Evenly distribute the *Sample* into 100 mL of boiling water and stir the mixture using a magnetic stirrer with a 25-mm long bar: a slurry is formed and the particles do not dissolve. Allow the slurry to cool to 5° and stir using a magnetic stirrer.
Acceptance criteria: A clear or slightly turbid solution occurs with its thickness dependent on the viscosity grade.
- **C.**
Solution A: Sulfuric acid and water (9 in 10). [NOTE—Carefully add the sulfuric acid to the water.]
Sample solution: 0.1 mL of the solution prepared for *Identification test B*
Analysis: To the *Sample solution* add 9 mL of *Solution A* and shake. Heat in a water bath for exactly 3 min, immediately cool in an ice bath, and add carefully 0.6 mL of ninhydrin TS. Shake and allow to stand at 25°.
Acceptance criteria: A red color develops immediately and it does not change to purple within 100 min.

D.

Sample solution: 2–3 mL of the solution prepared for *Identification test B*

Analysis: Pour the *Sample solution* onto a glass slide as a thin film and allow the water to evaporate.

Acceptance criteria: A coherent, clear film forms on the glass slide.

E.

Sample solution: 50 mL of the solution prepared in *Identification test B*

Analysis: Add the *Sample solution* to exactly 50 mL of water in a beaker. Insert a thermometer into the solution. Stir the solution on a magnetic stirrer/hot plate, and begin heating at a rate of 2°–5°/min. Determine the temperature at which a turbidity increase begins to occur and designate this temperature as the flocculation temperature.

Acceptance criteria: The flocculation temperature is higher than 50°.

ASSAY

PROCEDURE

[CAUTION—Perform all steps involving *Hydriodic acid* carefully, in a well-ventilated hood. Use goggles, acid-resistant gloves, and other appropriate safety equipment. Be exceedingly careful when handling the hot vials because they are under pressure. In the event of hydriodic exposure, wash with copious amounts of water, and seek medical attention at once.]

Apparatus

Reaction vial: A 5-mL pressure-tight serum vial, 20 mm in outside diameter, 50 mm in height, and 20 mm in outside diameter and 13 mm in inside diameter at the mouth, equipped with a pressure-tight septum having a polytetrafluoroethylene-faced butyl rubber and an airtight seal using an aluminum crimp or any sealing system that provides sufficient airtightness

Heater: A heating module with a square-shaped aluminum block having holes 20 mm in diameter and 32 mm in depth, so that the reaction vial fits. The heating module is also equipped with a magnetic stirrer capable of mixing the contents of the vial, or a reciprocal shaker that performs a reciprocating motion approximately 100 times/min can be used.

Hydriodic acid: Use a reagent having a specific gravity of at least 1.69, equivalent to 55%–57% hydrogen iodide (HI).

Internal standard solution: 30 mg/mL of *n*-octane in *o*-xylene

Standard solution: Into a suitable serum vial weigh 60–100 mg of adipic acid, add 2.0 mL of *Hydriodic acid*, and then pipet 2.0 mL of the *Internal standard solution* into the vial. Close the vial securely with a suitable septum stopper. Weigh the vial and contents, add 45 µL of methyl iodide with a syringe through the septum, weigh again, and calculate the weight of methyl iodide added, by difference. Shake, and allow the layers to separate. Use the upper layer as the *Standard solution*.

Sample solution: Transfer 0.065 g of Methylcellulose to a 5-mL thick-walled reaction vial equipped with a pressure-tight septum closure, add 60–100 mg of adipic acid, and pipet 2.0 mL of the *Internal standard solution* into the vial. Cautiously pipet 2.0 mL of *Hydriodic acid* into the mixture, immediately secure the closure, and weigh accurately. Using the magnetic stirrer from the heating module, or using a reciprocal shaker, mix the contents of the vial continuously for 60 min while heating the block so that the temperature of the contents is maintained at $130 \pm 2^\circ$. If a reciprocal shaker or magnetic stirrer cannot be used, shake the vial well by hand at 5-min intervals during the initial 30 min of the heating time. Allow the vial to cool, and weigh again. If the weight loss is less than 0.50% of the contents and there is no evidence of a leak, use the upper layer of the mixture as the *Sample solution*.

Chromatographic system**Mode:** GC**Detector:** Thermal conductivity or hydrogen flame ionization**Column:** 3- to 4-mm \times 1.8- to 3-m; packed with 10%-20% liquid phase G1, 125–150 μ m in diameter on 100- to 120-mesh support S1A. [NOTE—Use a column giving well-resolved peaks of methyl iodide and the internal standard, in that order.]**Column temperature:** 100°**Carrier gas:** Helium for the thermal conductivity detector, and helium or nitrogen for the hydrogen flame ionization detector**Flow rate:** Adjust so that the retention time of the internal standard is about 10 min.**Injection volume:** 1 or 2 μ L**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of methoxy in the portion of Methylcellulose taken:

$$\text{Result} = X \times (R_U/R_S) \times (W_S/W)$$

 X = ratio of the formula weights of methoxy to methyl iodide times 100%, 21.864 R_U = ratio of the peak area of methyl iodide to that of the internal standard from the *Sample solution* R_S = ratio of the peak area of methyl iodide to that of the internal standard from the *Standard solution* W_S = weight of methyl iodide in the *Standard solution* (mg) W = weight of Methylcellulose, calculated on the dried basis, taken for the *Assay* (mg)**Acceptance criteria:** 26.0%–33.0% calculated on the dried basis**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 1.5%

Delete the following:

- **HEAVY METALS** (231), *Method III*

Analysis: For the *Standard Preparation*, add the *Standard Lead Solution* before digestion. Omit the *Monitor Preparation*.**Acceptance criteria:** NMT 20 ppm; the color of the test solution is not darker than that of the control solution.

● (Official 1-Jan-2018)

SPECIFIC TESTS

- **LOSS ON DRYING** (731)

Analysis: Dry at 105° for 1 h.**Acceptance criteria:** NMT 5.0%

- **VISCOSITY—CAPILLARY METHODS** (911) and **VISCOSITY—ROTATIONAL METHODS** (912)

[NOTE—The density is 1.00 g/mL, so there is no necessity for determining the density at every measurement in the case of having the confirmation data.]

Method 1: This method is applied to samples with a viscosity of less than 600 mPa \cdot s. Weigh a quantity of Methylcellulose, equivalent to 4.000 g, calculated on the dried basis, transfer into a wide-mouth bottle, and add hot water (90°–99°) to obtain the total weight of the sample and water of 200.0 g. Cap the bottle and stir by mechanical means at 400 \pm 50 rpm for 10–20 min until particles are thoroughly dispersed and wetted out. Scrape down the walls of the bottle with a spatula, if necessary, to ensure that there is no undissolved material on the sides of the bottle. Continue the stirring in

a cooling water bath equilibrated at a temperature below 5° for another 20–40 min. Adjust the solution weight, if necessary, to 200.0 g using cold water. Centrifuge the solution, if necessary, to expel any entrapped air bubbles. Using a spatula, remove any foam, if present. Perform the test with this solution at 20 \pm 0.1° to obtain the kinematic viscosity (ν). Separately, determine the density (ρ) of the solution, and calculate the viscosity (η) as $\eta = \rho\nu$.

Method 2: This method is applied to samples with a viscosity of 600 mPa \cdot s or higher. Weigh a quantity of Methylcellulose, equivalent to 10.00 g, calculated on the dried basis, transfer into a wide-mouth bottle, and add hot water (90°–99°) to obtain the total weight of the sample and water of 500.0 g. Capping the bottle, stir by mechanical means at 400 \pm 50 rpm for 10–20 min until particles are thoroughly dispersed and wetted out. Scrape down the walls of the bottle with a spatula, if necessary, to ensure that there is no undissolved material on the sides of the bottle. Continue the stirring in a cooling water bath equilibrated at a temperature below 5° for another 20–40 min. Adjust the solution weight, if necessary, to 500.0 g using cold water. Centrifuge the solution, if necessary, to expel any entrapped air bubbles. Using a spatula, remove any foam, if present. Determine the viscosity of this solution at 20 \pm 0.1° using a single-cylinder type rotational viscometer.

Apparatus: Brookfield type LV model or equivalent. For rotor no., revolution, and calculation multiplier, apply the conditions specified in *Table 1*.

Table 1

Labeled Viscosity ^a (mPa \cdot s)	Rotor No.	Revolution (rpm)	Calculation Multiplier
600 or more and less than 1400	3	60	20
1400 or more and less than 3500	3	12	100
3500 or more and less than 9500	4	60	100
9500 or more and less than 99,500	4	6	1000
99,500 or more	4	3	2000

^a The Labeled Viscosity is based on the manufacturer's specifications.

Operation of apparatus: Allow the spindle to rotate for 2 min before taking the measurement. Allow a rest period of at least 2 min between subsequent measurements. Repeat the operation to rotate the spindle specified above twice, and average the three readings.

Acceptance criteria: 80.0%–120.0% of that stated on the label for viscosity types less than 600 mPa \cdot s, and 75.0%–140.0% of that stated on the label for viscosity types 600 mPa \cdot s or higher

- **pH** (791)

Analysis: Measure the pH of the solution prepared in the test for *Viscosity*. Read the indicated pH value after the probe has been immersed for 5 \pm 0.5 min.

Acceptance criteria: 5.0–8.0**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate its nominal viscosity type [viscosity of a solution (1 in 50)] in milli-Pascal seconds (mPa \cdot s).

Methylcellulose Ophthalmic Solution

» Methylcellulose Ophthalmic Solution is a sterile solution of Methylcellulose. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of methylcellulose. It may contain suitable antimicrobial, buffering, and stabilizing agents.

Packaging and storage—Preserve in tight containers.

Identification—

A: Heat a few mL of Ophthalmic Solution: the solution becomes cloudy and a flaky precipitate, which redissolves as the solution cools, appears.

B: Pour a few mL of Ophthalmic Solution onto a glass plate, and allow the water to evaporate: a thin, self-sustaining film results.

Sterility Tests (71): meets the requirements.

pH (791): between 6.0 and 7.8.

Assay—To boiling flask A, as described under *Methoxy Determination* (431), pipet a quantity of Ophthalmic Solution, equivalent to 50 mg of methylcellulose. Evaporate on a steam bath to dryness, cool the flask in an ice bath, add the specified amount of hydriodic acid, and proceed as directed under *Methoxy Determination* (431). Each mL of 0.1 N sodium thiosulfate is equivalent to 1.753 mg of methylcellulose.

Methylcellulose Oral Solution

» Methylcellulose Oral Solution is a flavored solution of Methylcellulose. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of methylcellulose.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat. Avoid freezing.

Identification—

A: Heat a few mL of Oral Solution: the solution becomes cloudy and a flaky precipitate, which redissolves as the solution cools, appears.

B: Pour a few mL of Oral Solution onto a glass plate, and allow the water to evaporate: a thin, self-sustaining film results.

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for the absence of *Escherichia coli*.

Alcohol Determination, Method II (611): between 3.5% and 6.5% of C_2H_5OH .

Assay—To boiling flask A, as described under *Methoxy Determination* (431), transfer an accurately measured volume of Oral Solution, equivalent to 50 mg of methylcellulose. Evaporate on a steam bath to dryness, cool the flask in an ice bath, add the specified amount of hydriodic acid, and proceed as directed under *Methoxy Determination* (431). Each mL of 0.1 N sodium thiosulfate is equivalent to 1.753 mg of methylcellulose.

Methylcellulose Tablets

» Methylcellulose Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methylcellulose.

Packaging and storage—Preserve in well-closed containers.

Identification—

A: Gently add about 250 mg of the residue obtained in the Assay to the top of 25 mL of water in a beaker, and allow to disperse over the surface, tapping the top of the container to ensure an even dispersion of the test specimen. Allow the beaker to stand until the specimen becomes transparent and mucilaginous (about 5 hours), swirl the beaker to wet the remaining substance, add a stirring bar, and stir until dissolved: the mixture remains stable when an equal volume of 1 N sodium hydroxide or 1 N hydrochloric acid is added.

B: Heat a few mL of the solution prepared for Identification test A: the solution becomes cloudy, and a flaky precipitate, which redissolves as the solution cools, appears.

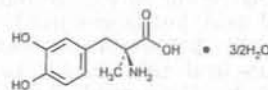
C: Pour a few mL of the solution prepared for Identification test A onto a glass plate, and allow the water to evaporate: a thin, self-sustaining film results.

Disintegration (701): 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 500 mg of methylcellulose, and transfer to a tared, fine fritted-glass, low-form, 30-mL crucible having a fitted crucible lid. Add 20 mL of alcohol, and macerate the solid for about 5 minutes, mixing intermittently with a glass stirring rod. Repeat the extraction with ten consecutive 10-mL portions of alcohol. Test for completeness of extraction by evaporating the last alcohol extract on a steam bath to dryness, taking up the residue in about 1 mL of water, and adding this to 5 mL of hot alkaline cupric tartrate TS (no red precipitate of cuprous oxide is formed within 5 minutes). If a precipitate is formed, continue with the alcohol extractions until the test is negative. Wash the completely extracted residue with a 10-mL portion of ether, using suction to drain off the liquid. Dry the residue in the crucible in a drying oven at 105° to constant weight. Weigh the crucible with the crucible lid in place. The weight of residue is the weight of methylcellulose present in the portion of powdered Tablets taken.

Methylropa



$C_{10}H_{13}NO_4 \cdot 1\frac{1}{2}H_2O$ 238.24

$C_{10}H_{13}NO_4$ 211.22

L-Tyrosine, 3-hydroxy- α -methyl-, sesquihydrate;
L-3-(3,4-Dihydroxyphenyl)-2-methylalanine sesquihydrate
[41372-08-1].

Anhydrous [555-30-6].

DEFINITION

Methylropa contains NLT 98.0% and NMT 101.0% of $C_{10}H_{13}NO_4$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)
Analytical wavelength: 280 nm
Sample solution: 40 µg/mL in 0.1 N hydrochloric acid
Acceptance criteria: Absorptivities, calculated on the anhydrous basis, do not differ by more than 3.0%.
- **C.**
Sample: 10 mg
Analysis: To the Sample add 0.15 mL of a solution of ninhydrin in sulfuric acid (1 in 250); a dark purple color is produced within 5–10 min. Add 0.15 mL of water.
Acceptance criteria: The color changes to pale brownish yellow.

ASSAY

- **PROCEDURE**
Sample solution: 200 mg of Methyldopa in 25 mL of glacial acetic acid, with the aid of heat. Cool to room temperature, and add 0.1 mL of crystal violet TS and 50 mL of acetonitrile.
Analysis: Titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 21.12 mg of $C_{10}H_{13}NO_4$.
Acceptance criteria: 98.0%–101.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS**, Method II (231): NMT 10 ppm (Official 1: Jan-2018)

• **LIMIT OF 3-O-METHYLMETHYLDOPA**

- Standard solution: 100 µg/mL of USP 3-O-Methylmethyldopa RS in methanol
Sample solution: 10 mg/mL of Methyldopa in methanol
Chromatographic system
(See *Chromatography* (621), *Thin-Layer Chromatography*.)
Mode: TLC
Developing solvent system: Butyl alcohol, glacial acetic acid, and water (65:15:25). [NOTE—Prepare this mixture fresh.]
Adsorbent: Thin-layer chromatographic plate with a suitable grade of cellulose, 250-µm thick, prewashed with the *Developing solvent system*. Wash the plate by placing it in a tank containing the *Developing solvent system*, and allowing the solvent to rise to the top of the plate. Dry with the aid of a current of dry air.
Solution A: 3 mg/mL of *p*-nitroaniline in 10 N hydrochloric acid. [NOTE—Prepare fresh solution, just before use.]
Solution B: 50 mg/mL of sodium nitrite. [NOTE—Prepare fresh solution, just before use.]
Spray reagent 1: Solution A and Solution B (9:1). [NOTE—Prepare fresh solution, just before spraying.]
Spray reagent 2: 250 mg/mL of sodium carbonate

Analysis

Samples: Standard solution and Sample solution
Apply 20 µL of the Sample solution in two 10-µL increments and 10 µL of the Standard solution so that the spots are NMT 0.5 cm in diameter. Develop using the *Developing solvent system* until the solvent front has moved about 10 cm from the origin. Remove the plate, and dry with a current of dry air until no odor of acetic acid is perceptible. Place the plate in a vertical position, and evenly spray with *Spray reagent 1* until the adsorbent layer is uniformly soaked down to the glass (do not overspray). Place the plate in a hori-

zontal position, and dry as completely as possible with a current of warm dry air (no odor of hydrochloric acid is perceptible). Place the plate in a vertical position, and evenly spray with *Spray reagent 2* until the plate is uniformly wet (do not overspray). The major methyldopa spot is black on a pale pink or orange background at an R_f value of 0.50, and the 3-O-methylmethyldopa spot is dark on a similar background at an R_f value of 0.65.

Acceptance criteria: The area and intensity of any 3-O-methylmethyldopa spot from the Sample solution are NMT those from the Standard solution (0.5%).

SPECIFIC TESTS

- **OPTICAL ROTATION**, *Specific Rotation* (781S): -25° to -28°
Sample solution: 44 mg/mL in a solvent that is a solution of aluminum chloride in water (2 in 3) that previously has been treated with activated charcoal, filtered, and adjusted with 0.25 N sodium hydroxide to a pH of 1.5
- **ACIDITY**: Dissolve 1.0 g in carbon dioxide-free water with the aid of heat, add 1 drop of methyl red TS, and titrate with 0.10 N sodium hydroxide to a yellow endpoint: NMT 0.50 mL is required.
- **WATER DETERMINATION**, Method I (921): 10.0%–13.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
USP Methyldopa RS
USP 3-O-Methylmethyldopa RS

Methyldopa Oral Suspension

» Methyldopa Oral Suspension is an aqueous suspension of Methyldopa. It contains one or more suitable flavors, wetting agents, and preservatives, and it may contain Sucrose. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{10}H_{13}NO_4$.

Packaging and storage—Preserve in tight, light-resistant containers, and store at a temperature not exceeding 26° .

USP Reference standards (11)—

USP Methyldopa RS

Identification—Apply 10-µL portions of the *Assay preparation* and the *Standard preparation* prepared as directed in the *Assay* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow to dry, develop the chromatogram in a solvent system consisting of equal volumes of acetone, butyl alcohol, glacial acetic acid, toluene, and water until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots by staining the plate with iodine vapor for about 50 minutes, then view the plate under short-wavelength UV light: the R_f value of the principal spot obtained from the *Assay preparation* corresponds to that obtained from the *Standard preparation*.

Uniformity of dosage units (905)—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698)—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

pH (791): between 3.0 and 5.0; between 3.2 and 3.8 if sucrose is present.

Assay—

Mobile phase—To 6.8 g of monobasic potassium phosphate add 750 mL of water, and stir until solution is complete. Adjust with 1 M phosphoric acid to a pH of 3.5, dilute with water to 1000 mL, mix, and pass through a filter having a 10- μ m or finer porosity.

Standard preparation—Dissolve an accurately weighed quantity of USP Methylidopa RS in 0.1 N sulfuric acid to obtain a solution having a known concentration of about 1 mg of anhydrous methylidopa per mL.

Assay preparation—Transfer an accurately measured volume of Oral Suspension, freshly mixed, equivalent to about 250 mg of methylidopa, to a 250-mL volumetric flask, dilute with 0.1 N sulfuric acid to volume, and mix to dissolve the methylidopa. Pass the solution through a 0.45- μ m membrane filter before using.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-mm detector and a 3.9-mm \times 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph three replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{10}H_{13}NO_4$ in each mL of the Oral Suspension taken by the formula:

$$250(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Methylidopa RS in the *Standard preparation*; V is the volume, in mL, of Oral Suspension taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methylidopa Tablets

DEFINITION

Methylidopa Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methylidopa ($C_{10}H_{13}NO_4$).

IDENTIFICATION

A.

Analysis: To about 10 mg of finely ground Tablets add 3 drops of a solution of ninhydrin in sulfuric acid (1 in 250).

Acceptance criteria: A dark purple color is produced within 5–10 min that changes to pale brownish yellow on addition of 3 drops of water.

B.

Analysis: To about 10 mg of finely ground Tablets add 2 mL of 0.1 N sulfuric acid and 2 mL of *Solution A*, prepared as directed in the *Assay*, followed by 0.25 mL of 6 N ammonium hydroxide.

Acceptance criteria: A dark purple color is produced immediately.

ASSAY

PROCEDURE

Solution A: Freshly dissolve 1 g of ferrous sulfate, 2 g of potassium sodium tartrate, and 100 mg of sodium bisulfite in water to make a 100-mL solution.

Solution B: 50 g/L of ammonium acetate in 20% alcohol. Adjust with 6 N ammonium hydroxide to a pH of 8.5.

Standard solution: 1 mg/mL of anhydrous methylidopa from USP Methylidopa RS in 0.1 N sulfuric acid

Sample solution: Nominally 1 mg/mL of methylidopa in 0.1 N sulfuric acid from NLT 20 powdered Tablets prepared as follows. To a 100-mL volumetric flask add 0.1 N sulfuric acid to fill about 50% of the volume of the flask, agitate by mechanical means for 15 min, and then dilute with 0.1 N sulfuric acid to volume. Filter the solution, and reject the first 20 mL of the filtrate.

Blank: Water

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 520 nm

Cell: 1 cm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*
Pipet 5 mL each of *Standard solution*, *Sample solution*, and *Blank* into separate 100-mL volumetric flasks. Add to each flask 5 mL of *Solution A*, and dilute with *Solution B* to volume.

Calculate the percentage of the labeled amount of methylidopa ($C_{10}H_{13}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Methylidopa RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methylidopa in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 20 min

Instrumental conditions

Mode: UV

Analytical wavelength: 280 nm

Standard solution: USP Methylidopa RS in *Medium*

Sample solution: Sample per *Dissolution* (711). Dilute with *Medium* to a concentration similar to that of the *Standard solution*.

Analysis: Determine the amount of methylidopa ($C_{10}H_{13}NO_4$) dissolved.

Tolerances: NLT 80% (Q) of the labeled amount of methylidopa ($C_{10}H_{13}NO_4$) is dissolved.

UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**
USP Methylidopa RS

Methylidopa and Chlorothiazide Tablets

» Methylidopa and Chlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of methylidopa ($C_{10}H_{13}NO_4$) and chlorothiazide ($C_7H_6ClN_3O_4S_2$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Chlorothiazide RS

USP Methyldopa RS

Identification—Transfer the finely ground contents of 1 Tablet to a test tube, add 10 mL of dilute alcohol (1 in 2), shake for 5 minutes, and centrifuge. Use the clear supernatant as the Test solution. Prepare a solution of alcohol and 0.1 N sodium hydroxide (1:1) containing in each mL about 10 mg of USP Methyldopa RS and 10 mg of USP Chlorothiazide RS. Apply 20 μ L of the Test solution on a line parallel to and about 2 cm from the bottom edge of a 20-cm \times 10-cm thin-layer chromatographic plate (see *Chromatography* (621)) coated with chromatographic silica gel mixture, and apply 20 μ L of the Standard solution separately on the starting line. Allow the spots to dry, develop the chromatogram in a solvent system consisting of equal volumes of glacial acetic acid, acetone, butyl alcohol, toluene, and water until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing tank, and allow the solvent to evaporate. View the plate under short-wavelength UV light: the solution under test exhibits two major spots having R_f values corresponding to those of the two major spots obtained with the Standard solution.

Dissolution (711)—

PROCEDURE FOR METHYLDOPA—

Medium: 0.1 N hydrochloric acid; 900 mL.**Apparatus 2:** 75 rpm.**Time:** 30 minutes.

Standard preparation—Dissolve an accurately weighed quantity of USP Methyldopa RS in *Medium*, and dilute quantitatively with the same solvent to obtain a solution having a known concentration of about 275 μ g of anhydrous methyldopa per mL.

Ferrous tartrate solution—Dissolve 1 g of ferrous sulfate, 2 g of potassium sodium tartrate, and 100 mg of sodium bisulfite in water to make 100 mL, and mix. Use a freshly prepared solution.

Buffer solution—Dissolve 50 g of ammonium acetate in 1000 mL of dilute alcohol (1 in 5). Adjust with 6 N ammonium hydroxide to a pH of 8.5.

Procedure—Filter 35 mL of the solution under test, and transfer an aliquot estimated to contain between 2 mg and 3 mg of methyldopa to a 100-mL volumetric flask. Adjust the final volume, if necessary, with *Medium* to 10 mL. To a second 100-mL volumetric flask add 10.0 mL of *Standard preparation*, and to a third 100-mL volumetric flask add 10.0 mL of *Medium* to provide a blank. Pipet 5.0 mL of *Ferrous tartrate solution* into each flask, dilute with *Buffer solution* to volume, and mix. Concomitantly determine the absorbances of the treated *Standard preparation* and test solution at the wavelength of maximum absorbance at about 520 nm, with a suitable spectrophotometer, against the reagent blank. Calculate the amount of $C_{10}H_{13}NO_4$ dissolved, in mg, taken by the formula:

$$9(C/V)(A_U/A_S)$$

in which C is the concentration, in μ g of anhydrous methyldopa per mL, of USP Methyldopa RS in the *Standard preparation*; V is the volume, in mL, of the aliquot of test solution used; and A_U and A_S are the absorbances of the solutions from the test solution and the *Standard preparation*, respectively.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{10}H_{13}NO_4$ is dissolved in 30 minutes.

PROCEDURE FOR CHLOROTHIAZIDE—

Medium: 0.05 M, pH 8.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) containing sodium sulfite (1 in 5000); 900 mL.

Apparatus 2: 75 rpm.**Time:** 60 minutes.

Procedure—Determine the amount of $C_7H_6ClN_3O_4S_2$ dissolved from UV absorbances of the solution under test, suitably diluted with *Medium*, if necessary, at the wavelength of maximum absorbance at about 317 nm in comparison with a Standard solution having a known concentration of USP Chlorothiazide RS in the same *Medium*.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_7H_6ClN_3O_4S_2$ is dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements with respect to methyldopa and to chlorothiazide.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 0.08 M monobasic sodium phosphate and methanol (95:5). Adjust by the addition of phosphoric acid to a pH of 2.8. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Transfer to a 100-mL volumetric flask accurately weighed quantities of USP Methyldopa RS and USP Chlorothiazide RS, equivalent to one-fifth of their labeled amounts, in mg, per Tablet. Add 15 mL of water and 5 mL of 1 N hydrochloric acid, and sonicate for about 3 minutes. Add 10 mL of acetonitrile, and sonicate for 2 minutes. Dilute with water to volume, and mix.

Assay preparation—Weigh and finely powder not less than 10 Tablets. Transfer an accurately weighed portion of the powder, equivalent to the weight of 1 Tablet, to a 500-mL volumetric flask. Add 75 mL of water and 25 mL of 1 N hydrochloric acid, and sonicate for about 5 minutes. Add 50 mL of acetonitrile, and sonicate for 10 minutes. Dilute with water to volume, and mix. Filter through a 0.45- to 2.0- μ m membrane filter, discarding the first 10 mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm \times 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the chlorothiazide peak is not less than 1300 theoretical plates, the tailing factor for chlorothiazide peak is not more than 2, the resolution, R , between the chlorothiazide and methyldopa peaks is not less than 7, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 1.0 for methyldopa and 2.5 for chlorothiazide. Calculate the quantity, in mg, of chlorothiazide ($C_7H_6ClN_3O_4S_2$) in the portion of Tablets taken by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorothiazide RS in the *Standard preparation*; and r_U and r_S are the peak responses of the chlorothiazide peak obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of methyldopa ($C_{10}H_{13}NO_4$) taken by the same formula, reading "methyldopa" instead of "chlorothiazide."

Methyldopa and Hydrochlorothiazide Tablets

DEFINITION

Methyldopa and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of methyldopa ($C_{10}H_{13}NO_4$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

- A.** The retention times of the two major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

- B.**

Analysis: To 10 mg of methyldopa from a portion of crushed Tablets add 0.15 mL of a solution of ninhydrin in sulfuric acid (1 in 250).

Acceptance criteria: A dark purple color is produced within 5–10 min. The color changes to pale brownish yellow upon adding 0.15 mL of water.

ASSAY

PROCEDURE

Buffer: 11.04 g/L of monobasic sodium phosphate. Initially add 950 mL of water, adjust with phosphoric acid to a pH of 2.8, and then dilute with water to volume.

Mobile phase: Methanol and *Buffer* (5:95)

Standard solution: Transfer a suitable quantity of USP Methyldopa RS to a suitable volumetric flask to prepare a 1-mg/mL solution of anhydrous methyldopa. Add a quantity of USP Hydrochlorothiazide RS that corresponds to the ratio of hydrochlorothiazide to methyldopa in the Tablets. Dissolve in 25% of the total volume a mixture of water, acetonitrile, and 1 N hydrochloric acid (1:1:0.5). Dilute with water to volume.

Sample solution: Transfer an equivalent to 250 mg of methyldopa (NLT 20 Tablets) to a 250-mL volumetric flask, and add 50 mL of water, 25 mL of acetonitrile, and 13 mL of 1 N hydrochloric acid. Shake the flask for 5 min, and dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 3.9-mm × 30.0-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—Adjust the flow rate to obtain relative retention times of about 0.38 and 1.0 for methyldopa and hydrochlorothiazide, respectively.]

Suitability requirements

Resolution: NLT 6.0 between methyldopa and hydrochlorothiazide

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methyldopa from the *Sample solution*

r_S = peak response of methyldopa from the *Standard solution*

C_S = concentration of USP Methyldopa RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methyldopa in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of hydrochlorothiazide from the *Sample solution*

r_S = peak response of hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Methyldopa

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Solution A: 10 mg/mL of ferrous sulfate, 20 mg/mL of potassium sodium tartrate, and 1 mg/mL of sodium bisulfite in water. [NOTE—Use a freshly prepared solution.]

Solution B: 50 mg/mL of ammonium acetate in dilute alcohol (1 in 5). Adjust with 6 N ammonium hydroxide to a pH of 8.5.

Standard solution: 0.275 mg/mL of anhydrous methyldopa from USP Methyldopa RS in *Medium*

Sample solution: Filter 35 mL of the solution under test through paper.

Instrumental conditions

Mode: Vis

Analytical wavelength: 520 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Transfer an aliquot of the *Sample solution* estimated to contain 2–3 mg of methyldopa to a 100-mL volumetric flask. Adjust the final volume, if necessary, with *Medium* to 10 mL. To a second 100-mL volumetric flask add 10.0 mL of *Standard solution*, and to a third 100-mL volumetric flask add 10.0 mL of *Medium* to provide a blank. Pipet 5.0 mL of *Solution A* into each flask, dilute with *Solution B* to volume, and mix. Determine the absorbances of the *Standard solution* and the *Sample solution* at the wavelength of maximum absorbance, using the blank in the reference cell. Calculate the percentage of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of anhydrous methyldopa in the *Standard solution* (µg/mL)

L = label claim (mg/Tablet)

V = volume of the medium, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$) is dissolved.

Hydrochlorothiazide

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 60 min

Instrumental conditions

Mode: Vis

Analytical wavelength: 317 nm

Cell: 1 cm

Standard solution: USP Hydrochlorothiazide RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* to a concentration that is similar to the *Standard solution*.
Tolerances: NLT 80% (Q) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905)**

Procedure for content uniformity: Proceed as directed in the *Assay*, except use the following *Sample solution*.

Sample solution: Transfer 1 Tablet to a 250-mL volumetric flask, add 50 mL of water, and shake gently, if necessary, to disintegrate the Tablet. Do not sonicate. After the Tablet has completely disintegrated, add 25 mL of acetonitrile, and shake by mechanical means for 30 min. Add 13 mL of 1 N hydrochloric acid, and shake by mechanical means for an additional 5 min. Dilute with water to volume.

Acceptance criteria: Meet the requirements with respect to methyldopa and hydrochlorothiazide

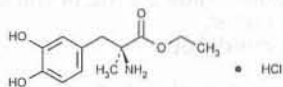
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**

USP Hydrochlorothiazide RS
 USP Methyldopa RS

Methyldopate Hydrochloride



$C_{12}H_{17}NO_4 \cdot HCl$ 275.73

L-Tyrosine, 3-hydroxy- α -methyl-, ethyl ester, hydrochloride.
 L-3-(3,4-Dihydroxyphenyl)-2-methylalanine ethyl ester hydrochloride [5123-53-5; 2508-79-4].

» Methyldopate Hydrochloride contains not less than 98.0 percent and not more than 101.0 percent of $C_{12}H_{17}NO_4 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards (11)—

USP Methyldopate Hydrochloride RS

Identification—

A: Infrared Absorption (197M).

B: Ultraviolet Absorption (197U)—

Solution: 50 μ g per mL.

Medium: 0.1 N hydrochloric acid.

Absorptivities at 280 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: It responds to *Identification test C* under *Methyldopa*.

D: It responds to the tests for *Chloride* (191), except that on the addition of the slight excess of 6 N ammonium hydroxide a brown precipitate is formed.

Specific rotation (781S): between -13.5° and -14.9° ($\lambda = 405$ nm).

Test solution: 40 mg per mL, in 0.1 N hydrochloric acid.

pH (791): between 3.0 and 5.0, in a solution (1 in 100).

Loss on drying (731)—Dry it at 100° and at a pressure not exceeding 5 mm of mercury for 2 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals, Method II (231):** 0.001%. • (Official 1-Jan-2018)

Assay—

Mobile solvent—Prepare a suitable solution of 0.02 M monobasic sodium phosphate and 0.015 M phosphoric acid in a water and methanol solution (approximately 15.5:4.5) such that the retention time of methyldopate hydrochloride is approximately 6.5 minutes.

Standard preparation—Dissolve an accurately weighed quantity of USP Methyldopate Hydrochloride RS in the *Mobile solvent* to obtain a solution containing about 1 mg per mL.

Assay preparation—Transfer about 50 mg of Methyldopate Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile solvent* to volume.

Procedure—Introduce separately 20- μ L portions of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* (621)) operated at 25°, by means of a suitable microsyringe or sampling valve, adjusting the operating parameters such that the peak obtained with the *Standard preparation* is 100% full-scale. Typically, the apparatus is fitted with a 4-mm \times 30-cm column that contains packing L1, is equipped with an UV detector capable of monitoring absorption at 280 nm and a suitable recorder, and is capable of operating at a column pressure between 700 and 1700 psi. In a suitable chromatogram, three replicate injections of the *Standard preparation* show a relative standard deviation of not more than 1.5%. Determine the peak areas, at equivalent retention times, obtained with the *Assay preparation* and the *Standard preparation*, and calculate the quantity, in mg, of $C_{12}H_{17}NO_4 \cdot HCl$ in the portion of Methyldopate Hydrochloride taken by the formula:

$$50C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Methyldopate Hydrochloride RS in the *Standard preparation*; and A_U and A_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methyldopate Hydrochloride Injection

» Methyldopate Hydrochloride Injection is a sterile solution of Methyldopate Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methyldopate hydrochloride ($C_{12}H_{17}NO_4 \cdot HCl$).

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.

USP Reference standards (11)—

USP Endotoxin RS

USP Methyldopate Hydrochloride RS

Identification—

A: Dilute a volume of Injection with a mixture of chloroform and methanol (1:1), if necessary, to obtain a solution containing about 5 mg of methyldopate hydrochloride per mL. Apply separately 10 μ L of this solution and 10 μ L of a *Standard solution* of USP Methyldopate Hydrochloride RS in a solvent mixture of chloroform and methanol (1:1) containing 5 mg per mL to a suitable thin-layer chromatographic

plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a saturated chamber with a solvent system consisting of a mixture of butyl alcohol, water, and formic acid (7:2:1), until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with Folin-Ciocalteu phenol TS followed by spraying with sodium carbonate solution (1 in 10); the R_f value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

B: It responds to the tests for *Chloride* (191), with the exception that the 6 N ammonium hydroxide is omitted.

Bacterial Endotoxins Test (85)—It contains not more than 0.5 USP Endotoxin Unit per mg of methyldopate hydrochloride.

pH (791): between 3.0 and 4.2.

Particulate Matter in Injections (788): meets the requirements under small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Buffer solution—To 214 g of monobasic potassium phosphate add 700 mL of water, and stir. Cautiously add 75 mL of sodium hydroxide solution (1 in 2), and stir until solution is complete. Adjust with sodium hydroxide solution (1 in 2) to a pH of 8.0, and dilute with water to 1000.0 mL.

Water-saturated tributyl phosphate—Shake 800 mL of tributyl phosphate with 100 mL of water, and discard the lower, aqueous phase. Filter the upper phase.

Standard preparation—Transfer about 25 mg of USP Methyldopate Hydrochloride RS, accurately weighed, to a 25-mL volumetric flask, add water to volume, and mix. Transfer 5 mL of this solution to a 100-mL volumetric flask, add 0.1 N sulfuric acid to volume, and mix. Use a freshly prepared solution. The *Standard preparation* contains about 50 µg per mL.

Assay preparation—Transfer to a 50-mL volumetric flask an accurately measured volume of Injection, equivalent to about 50 mg of methyldopate hydrochloride, add water to volume, and mix. Transfer a 5.0-mL aliquot of the solution to a 60-mL separator, add 15 mL of *Buffer solution* and 10 mL of *Water-saturated tributyl phosphate*, and shake for about 1 minute. Allow the phases to separate, and transfer the lower, aqueous phase to a second 60-mL separator. To this separator add a second 10-mL portion of *Water-saturated tributyl phosphate*, shake for about 1 minute, allow the phases to separate, discard the lower, aqueous phase, and add the upper tributyl phosphate phase to the phase retained in the first separator. Rinse the second separator with about 2 mL of *Water-saturated tributyl phosphate*, and add the rinsing to the first separator. Extract the phase contained in the first separator with two 25-mL portions of 0.1 N sulfuric acid. Collect the acid extracts in a 100-mL volumetric flask, add 0.1 N sulfuric acid to volume, and mix. Filter, if necessary, to obtain a clear solution.

Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 283 nm, with a suitable spectrophotometer, using 0.1 N sulfuric acid as the blank. Calculate the quantity, in mg, of methyldopate hydrochloride ($C_{12}H_{17}NO_4 \cdot HCl$) in each mL of the Injection taken by the formula:

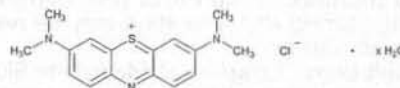
$$(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Methyldopate Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and A_U and A_S are

the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Methylene Blue

Change to read:



$C_{16}H_{18}ClN_3S \cdot xH_2O$ <small>▲USP40</small>	
Phenothiazin-5-ium, 3,7-bis(dimethylamino)-, chloride;	
▲3,7-Bis(dimethylamino)phenothiazin-5-ium chloride;	
Pentahydrate	409.93
[32680-41-4] <small>▲USP40</small>	
Trihydrate	▲373.90 <small>▲USP40</small>
[7220-79-3]	
▲Monohydrate	337.90
[122965-43-9] <small>▲USP40</small>	
Anhydrous	319.85
[61-73-4]	

DEFINITION

Change to read:

Methylene Blue contains ▲NLT 97.0% ▲USP40 and NMT 103.0% of methylene blue ($C_{16}H_{18}ClN_3S$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- ▲A ▲USP40 **INFRARED ABSORPTION** (197K)

Add the following:

- ▲B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲USP40

Add the following:

- ▲C. **IDENTIFICATION TESTS—GENERAL** (191), *Chloride*
Sample solution: Ignite 50 mg of Methylene Blue with 0.5 g of anhydrous sodium carbonate. Cool and dissolve the residue in 10 mL of 2 N nitric acid. Filter and use 2 mL of the filtrate for performing the test.
Acceptance criteria: Meets the requirements ▲USP40

ASSAY

Change to read:

• PROCEDURE

▲[NOTE—All solutions containing methylene blue are recommended to be prepared fresh before analysis.]

Solution A: 0.1% trifluoroacetic acid in water

Solution B: Acetonitrile

Diluent: *Solution A* and *Solution B* (70:30)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
25	30	70
32	30	70
33	80	20
38	80	20

Standard solution: 1 mg/mL of USP Methylene Blue RS in *Diluent*. Stirring and sonication may be necessary for complete dissolution.

Sample solution: 1 mg/mL of Methylene Blue in *Diluent*. Stirring and sonication may be necessary for complete dissolution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 246 nm

Column: 4.6-mm × 10-cm; 3.5-μm packing L11

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 3.0

Relative standard deviation: NMT 1.10%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylene blue

(C₁₆H₁₈ClN₃S) in the portion of Methylene Blue taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of methylene blue from the *Sample solution*

r_s = peak response of methylene blue from the *Standard solution*

C_s = concentration of USP Methylene Blue RS in the *Standard solution* (mg/mL)

C_u = concentration of Methylene Blue in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis[▲]USP40

IMPURITIES

Change to read:

- **RESIDUE ON IGNITION** (281): [▲]NMT 0.15%[▲]USP40

Change to read:

- [▲]**ARSENIC**

Analysis: Proceed as directed in *Elemental Impurities—Procedures* (233), *Procedure 2: ICP-MS*.[▲]USP40

Acceptance criteria: 8 ppm

Change to read:

- **COPPER OR ZINC**

Analysis: Proceed as directed in *Elemental Impurities—Procedures* (233), *Procedure 2: ICP-MS*.

Acceptance criteria: NMT 100 ppm of zinc and NMT 200 ppm of copper[▲]USP40

Change to read:

• ORGANIC IMPURITIES

[▲]**Diluent, Mobile phase, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 1 mg/mL of USP Methylene Blue RS and 0.025 mg/mL of USP Azure B RS in *Diluent*

Sensitivity solution: 0.5 μg/mL of USP Methylene Blue RS in *Diluent* from the *Standard solution*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 3.5 between methylene blue and azure B peaks, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of azure B or any unspecified impurity in the portion of Methylene Blue taken:

$$\text{Result} = (r_u/r_t) \times 100$$

r_u = peak response of azure B or any unspecified impurity from the *Sample solution*

r_t = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See Table 2. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azure B ^a	0.8	2.5
Methylene blue	1.0	—
Any unspecified impurity	—	0.10
Total impurities ^b	—	0.5

^a 3-(Dimethylamino)-7-(methylamino)-phenothiazine-5-ium chloride.

^b Total impurities does not include azure B.

[▲]USP40

SPECIFIC TESTS

Change to read:

- **LOSS ON DRYING** (731)

Analysis: Dry at [▲]105° for 5 h.[▲]USP40

Acceptance criteria: [▲]8.0%–22.0%[▲]USP40

Add the following:

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 2.5 USP Endotoxin Units/mL[▲]USP40

Add the following:

- **MICROBIAL ENUMERATION TESTS** (61): The total aerobic microbial count is NMT 10² cfu/g.[▲]USP40

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in well-closed containers and protect from light. Store [▲]below 30°.[▲]USP40

Change to read:**• USP REFERENCE STANDARDS (11)**

- ▲ USP Azure B RS
- 3-(Dimethylamino)-7-(methylamino)-phenothiazine-5-ium chloride.
- $C_{15}H_{16}ClN_3S$ 305.82
- USP Endotoxin RS
- ▲ USP40
- USP Methylene Blue RS

Methylene Blue Injection

» Methylene Blue Injection is a sterile solution of Methylene Blue in Water for Injection. It contains, in each mL, not less than 9.5 mg and not more than 10.5 mg of methylene blue ($C_{16}H_{18}ClN_3S \cdot 3H_2O$).

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.

USP Reference standards (11)—

- USP Endotoxin RS
- USP Methylene Blue RS

Identification—

A: The visible absorption spectrum of the solution employed for measurement of absorbance in the Assay exhibits maxima and minima at the same wavelengths as that of the Standard solution employed in the Assay, concomitantly measured.

B: Dilute a portion of the Injection with an equal volume of methanol. Dissolve 5 mg of USP Methylene Blue RS in 1 mL of a mixture of equal volumes of methanol and water. Apply 1 μ L of each solution to a thin-layer chromatographic plate, coated with a 0.25-mm layer of chromatographic silica gel, allow the spots to dry, and develop the chromatogram, using a mixture of water, alcohol, and acetic acid (4:3:3) as the solvent system, until the solvent front has moved about 10 cm above the line of application. Remove the plate from the developing chamber, and allow the solvent to evaporate: the R_f value of the principal spot obtained from the Methylene Blue corresponds to that obtained from the Reference Standard.

Bacterial Endotoxins Test (85)—It contains not more than 2.5 USP Endotoxin Units per mL.

pH (791): between 3.0 and 4.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—Dilute an accurately measured volume of Injection, equivalent to about 100 mg of methylene blue trihydrate to a 200-mL volumetric flask, dissolve in and dilute with diluted alcohol to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with diluted alcohol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with diluted alcohol to volume, and mix. This solution contains about 2.4 μ g of methylene blue trihydrate (2 μ g of anhydrous methylene blue) per mL. Prepare a Standard solution of USP Methylene Blue RS as directed in the Assay under *Methylene Blue*. Concomitantly determine the absorbance of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 663 nm, with a suitable spectrophotometer, using diluted alcohol as the blank. Calculate the quantity, in mg, of

methylene blue ($C_{16}H_{18}ClN_3S \cdot 3H_2O$) in each mL of the Injection taken by the formula:

$$(373.90 / 319.86)(40C / V)(A_U / A_S)$$

in which 373.90 and 319.86 are the molecular weights of methylene blue trihydrate and anhydrous methylene blue, respectively; C is the concentration, in μ g per mL, of USP Methylene Blue RS in the Standard solution; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Methylene Blue Injection, Veterinary**DEFINITION**

Methylene Blue Injection, Veterinary is a sterile solution of Methylene Blue in Water for Injection. It contains NLT 9.5 mg/mL and NMT 10.5 mg/mL of methylene blue trihydrate ($C_{16}H_{18}ClN_3S \cdot 3H_2O$). Prepare Methylene Blue Injection, Veterinary as follows (see *Pharmaceutical Compounding—Sterile Preparations* (797)):

Methylene Blue	5 g
Sterile Water for Injection or Sodium Chloride Injection (0.9%), a sufficient quantity to make	500 mL

Dissolve an accurately weighed quantity of Methylene Blue in Sterile Water for Injection or Sodium Chloride Injection (0.9%), and dilute to volume, with mixing. Sterilize by a suitable means, such as sterile filtration or autoclaving.

IDENTIFICATION

- **A.** The visible absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*, concomitantly measured as directed in the Assay.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**
 - Diluent:** Methanol and water (1:1)
 - Standard solution:** 5 mg/mL of USP Methylene Blue RS in Diluent
 - Sample solution:** Dilute a portion of the Injection with an equal volume of methanol.
 - Application volume:** 1 μ L
 - Developing solvent system:** Alcohol, acetic acid, and water (3:3:4)
 - Analysis**
 - Samples:** *Standard solution* and *Sample solution*
 - Allow the spots to dry, and develop until the solvent front has moved about 10 cm above the line of application. Remove the plate from the chamber, and allow the solvent to evaporate.
 - Acceptance criteria:** The R_f value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.

ASSAY**• PROCEDURE**

- Standard solution:** 2 μ g/mL of USP Methylene Blue RS in diluted alcohol
- Sample solution:** Dilute a volume of Injection with diluted alcohol to obtain a solution with a nominal concentration of about 2.4 μ g of methylene blue trihydrate (2 μ g of anhydrous methylene blue)/mL.

Spectrometric conditions(See *Ultraviolet-Visible Spectroscopy* (857).)**Mode:** UV-Vis**Detector:** 663 nm**Cell:** 1 cm**Analysis**

Samples: *Standard solution* and *Sample solution*. Concurrently determine the absorbance of both solutions, using diluted alcohol as the blank. Calculate the quantity, in mg, of $C_{16}H_{18}ClN_3S \cdot 3H_2O$ in each mL of injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times L \times (M_{r1}/M_{r2})$$

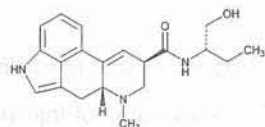
- A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of methylene blue trihydrate in the *Standard solution* (mg/mL)
 C_U = nominal concentration of methylene blue trihydrate in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of methylene blue trihydrate (373.90)
 M_{r2} = molecular weight of methylene blue anhydrous (319.86)
 L = labeled amount of methylene blue in the injection (10.0 mg/mL)
Acceptance criteria: 9.5–10.5 mg/mL of $C_{16}H_{18}ClN_3S \cdot 3H_2O$

SPECIFIC TESTS

- pH (791):** 3.0–4.5
- BACTERIAL ENDOTOXINS TEST (85):** NMT 0.17 USP Endotoxin Unit/mg of methylene blue
- STERILITY TESTS (71):** Meets the requirements
- OTHER REQUIREMENTS:** It meets the requirements under *Labeling (7)*, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I amber glass. Store at room temperature, protected from light.
- LABELING:** Label it to indicate that it is to be discarded after its beyond use date, to state that it is to be kept out of the reach of children, and to indicate the nominal content of methylene blue in the injection and whether it was prepared in Sterile Water for Injection or in Sodium Chloride Injection (0.9%). Label it to indicate that the dose is not to exceed 30 mg of methylene blue/kg of body weight/h. Label it to indicate that it is for veterinary use only and to state the *Beyond-Use Date*.
- BEYOND-USE DATE:** NMT 365 days after the date on which it was compounded
- USP REFERENCE STANDARDS (11)**
 USP Endotoxin RS
 USP Methylene Blue RS

Methylergonovine Maleate

$C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$ 455.50
 Ergoline-8-carboxamide, 9,10-didehydro-N-[1-(hydroxymethyl)propyl]-6-methyl-, [8 β (S)-, (Z)-2-butenedioate (1:1) (salt);
 9,10-Didehydro-N-[(S)-1-(hydroxymethyl)propyl]-6-methylethylergoline-8 β -carboxamide maleate (1:1) (salt) [57432-61-8].

DEFINITION

Methylergonovine Maleate contains NLT 97.0% and NMT 103.0% of methylergonovine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION

- A. INFRARED ABSORPTION (197K)**
- B.** The R_f values of the principal fluorescent spot and the principal blue spot of the *Sample solution* correspond to those of the *Standard stock solution*, as obtained in the test for *Related Alkaloids*.

ASSAY**PROCEDURE**

Conduct this procedure with a minimum exposure to light.

Mobile phase: Acetonitrile and 2.0 g/L of monobasic potassium phosphate (1:4)

Diluent: 2.5 mg/mL of tartaric acid prepared as follows. Transfer a suitable amount of tartaric acid to a suitable volumetric flask, add 50% of the flask volume of water, and mix with shaking. Dilute with methanol to volume. Allow the mixture to cool before use.

Standard stock solution: 0.1 mg/mL of USP Methylergonovine Maleate RS in *Diluent*. Shake by mechanical means for 15 min.

Standard solution: 4 μ g/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Sample stock solution: 0.2 mg/mL of Methylergonovine Maleate in *Diluent*. Shake by mechanical means for 15 min or until completely dissolved.

Sample solution: 4 μ g/mL of Methylergonovine Maleate from the *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Fluorescence with excitation at 315 nm and emission at 423 nm

Column: 4.6-mm \times 25-cm; packing L7

Temperature: 30°

Flow rate: 2 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylergonovine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$) in the portion of Methylergonovine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methylergonovine Maleate RS in the *Standard solution* (μ g/mL)

C_U = concentration of Methylergonovine Maleate in the *Sample solution* (μ g/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- RESIDUE ON IGNITION (281):** NMT 0.1%

RELATED ALKALOIDS

Conduct this test promptly, without exposure to daylight and with minimum exposure to artificial light. Solutions containing methylergonovine maleate should be prepared immediately before use.

Diluent: Alcohol and ammonium hydroxide (9:1)

Standard stock solution: 10 mg/mL of USP

Methylergonovine Maleate RS in *Diluent*

Standard solution A: 0.20 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Standard solution B: 0.10 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Standard solution C: 0.05 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Sample solution: 10 mg/mL of Methylergonovine Maleate in *Diluent*

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Developing solvent system: Chloroform, methanol, and water (75:25:3), equilibrated for 30 min

Spray reagent: 10 mg/mL of *p*-dimethylaminobenzaldehyde in a cooled mixture of alcohol and hydrochloric acid (1:1)

Analysis

Samples: *Standard stock solution*, *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Proceed as directed in the chapter. Locate the spots on the plate by spraying thoroughly and evenly with *Spray reagent*. Immediately dry in a stream of nitrogen for 2 min.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard stock solution*. Estimate the concentration of any other spots observed from the *Sample solution* by comparison with *Standard solution A*, *Standard solution B*, and *Standard solution C*. The spots from *Standard solution A*, *Standard solution B*, and *Standard solution C* are equivalent to 2.0%, 1.0%, and 0.50% of impurities, respectively. The sum of the impurities is NMT 2.0%.

SPECIFIC TESTS

• OPTICAL ROTATION (781S), *Specific Rotation*

Sample solution: 5 mg/mL of Methylergonovine Maleate in water

Acceptance criteria: +44° to +50°

• pH (791)

Sample solution: 0.2 mg/mL of Methylergonovine Maleate in water

Acceptance criteria: 4.4–5.2

• LOSS ON DRYING (731)

Analysis: Dry under vacuum at 80° to constant weight.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a cold place.

• USP REFERENCE STANDARDS (11)

USP Methylergonovine Maleate RS

Methylergonovine Maleate Injection

DEFINITION

Methylergonovine Maleate Injection is a sterile solution of Methylergonovine Maleate in Water for Injection. Each mL contains NLT 90.0% and NMT 110.0% of the labeled amount of methylergonovine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$).

IDENTIFICATION

• **A.** The R_f values of the principal fluorescent spot and the principal blue spot of the *Sample solution* correspond to those of the *Standard stock solution*, as obtained in the procedure for *Organic Impurities, Related Alkaloids*.

• B. PROCEDURE

Sample solution: 0.67 mg/mL of methylergonovine maleate from Injection in water

Analysis: The *Sample solution* exhibits a bluish fluorescence under UV light. To this solution add 2 mL of a solution of glacial acetic acid in ethyl acetate (1:2), and stratify 2 mL of sulfuric acid, by pipetting, under the solution.

Acceptance criteria: A bluish-purple ring appears at the interface of the two liquids.

ASSAY

• PROCEDURE

[NOTE—Conduct this procedure with minimum exposure to light.]

Mobile phase: Acetonitrile and 0.015 M monobasic potassium phosphate solution (1:4)

Diluent: 5 mg/mL of tartaric acid in water and methanol (1:1). Allow the mixture to cool before use. [NOTE—Dissolve tartaric acid with water, then add an equal volume of methanol.]

Standard solution: 100 μ g/mL of USP Methylergonovine Maleate RS in *Diluent*. [NOTE—Shake by mechanical means for 15 min or until completely dissolved.]

Sample solution: 100 μ g/mL of methylergonovine maleate from Injection in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4-mm \times 25-cm; packing L7

Column temperature: 30°

Flow rate: 2 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$ in each mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methylergonovine Maleate RS in the *Standard solution* (μ g/mL)

C_U = nominal concentration of the *Sample solution* (μ g/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Organic Impurities

• PROCEDURE: RELATED ALKALOIDS

[NOTE—Conduct this test promptly, without exposure to daylight and with minimum exposure to artificial light.]

Diluent: Alcohol and ammonium hydroxide (9:1)

[NOTE—All solutions should be prepared immediately before use.]

Standard stock solution: 10 mg/mL of USP Methylergonovine Maleate RS in *Diluent*

Standard solution A: 0.50 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Standard solution B: 0.20 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Standard solution C: 0.10 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Standard solution D: 0.05 mg/mL of USP Methylergonovine Maleate RS from the *Standard stock solution* in *Diluent*

Sample solution: Transfer the equivalent of 5 mg of methylergonovine maleate from Injection to a separator, and extract with three 5-mL portions of chloroform. Discard the chloroform extracts. Render alkaline to litmus with 6 N ammonium hydroxide, and extract with three 5-mL portions of chloroform. Evaporate the combined extracts with the aid of a current of air, but without heat, to dryness. Dissolve the residue so obtained in 0.5 mL of *Diluent*.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Developing solvent system: Chloroform, methanol, and water (75:25:3), equilibrated for 30 min

Spray reagent: 10 mg/mL of *p*-dimethylaminobenzaldehyde in a cooled mixture of alcohol and hydrochloric acid (1:1)

Analysis

Samples: *Standard stock solution*, *Standard solution A*, *Standard solution B*, *Standard solution C*, *Standard solution D*, and *Sample solution*
Proceed as directed in the chapter. Locate the spots on the plate by spraying thoroughly and evenly with *Spray reagent*. Immediately dry in a stream of nitrogen for 2 min.

Acceptance criteria: The R_f value of the principal spot from the *Sample solution* corresponds to that from the *Standard stock solution*. Estimate the concentration of any other spots observed in the lane for the *Sample solution* by comparison with *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Standard solution D*: the spots from the 0.50-, 0.20-, 0.10-, and 0.05-mg/mL dilutions are equivalent to 5.0%, 2.0%, 1.0%, and 0.50% of impurities, respectively. The sum of the impurities is NMT 5.0%.

SPECIFIC TESTS

- **pH (791):** 2.7–3.5
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 1.7 USP Endotoxin Units/ μ g of methylergonovine maleate
- **OTHER REQUIREMENTS:** Meets the requirements under *Injections and Implanted Drug Products* (1)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose, light-resistant containers, preferably of Type I glass. Store in a refrigerator.
- **USP REFERENCE STANDARDS (11)**
USP Endotoxin RS
USP Methylergonovine Maleate RS

Methylergonovine Maleate Tablets

» Methylergonovine Maleate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methylergonovine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—
USP Methylergonovine Maleate RS

Identification—

A: The R_f values of the principal fluorescent spot and the principal blue spot in the chromatogram of the *Test preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the test for *Related alkaloids* under *Ergonovine Maleate*, using the Tablets instead of Ergonovine Maleate.

B: Transfer a quantity of powdered Tablets, equivalent to about 4 mg of methylergonovine maleate, to a separator, add 20 mL of water, and render alkaline to litmus with sodium carbonate solution (1 in 10). Extract with three 20-mL portions of chloroform, filter the combined chloroform extracts into a small evaporating dish, and evaporate on a steam bath to dryness. Dissolve the residue in a mixture of 6 mL of water and 0.3 mL of hydrochloric acid, and filter, if necessary: the solution so obtained exhibits a bluish fluorescence under UV light. To this solution, add 2 mL of a solution of glacial acetic acid in ethyl acetate (1 in 2), and stratify 2 mL of sulfuric acid, by pipetting, under the solution: a bluish purple ring appears at the interface of the two liquids.

Dissolution (711)—

Medium: tartaric acid solution (1 in 200); 900 mL.

Apparatus 2: 75 rpm.

Time: 30 minutes.

Procedure—Filter a portion of the solution under test into a flask. Concomitantly determine the fluorescence intensity of this solution in comparison with a *Standard solution* of USP Methylergonovine Maleate RS in the same medium having a known concentration of about 0.22 μ g per mL in a fluorometer at an excitation wavelength of about 327 nm and an emission wavelength of about 428 nm, using tartaric acid solution (1 in 200) as the blank.

Tolerances—Not less than 70% (Q) of the labeled amount of $C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Related alkaloids—[NOTE—Conduct this test without exposure to daylight and with the minimum exposure to artificial light.]

Solvent mixture—Mix 75 volumes of chloroform, 25 volumes of methanol, and 1 volume of ammonium hydroxide.

Detecting reagent—Cautiously dissolve 800 mg of *p*-dimethylaminobenzaldehyde in a mixture of alcohol and sulfuric acid (101:11).

Test preparation—Transfer a quantity of finely powdered Tablets, equivalent to 5.0 mg of methylergonovine maleate, to a suitable container, add 50 mL of *Solvent mixture*, and stir with the aid of a magnetic stirrer for 40 minutes. Filter, rinsing the container with two 10-mL portions of *Solvent mixture*. Evaporate the combined filtrates in vacuum at 25° to 30°, and dissolve the residue in 2.0 mL of *Solvent mixture*.

Standard stock solution—Transfer 25 mg of USP Methylergonovine Maleate RS to a 10-mL volumetric flask, add *Solvent mixture* to volume, and mix to obtain a solution having a known concentration of 2.5 mg per mL.

Standard preparations A, B, C, and D—Dilute accurately measured volumes of *Standard stock solution* quantitatively with *Solvent mixture* (designated below as parts by volume of *Standard stock solution* in total parts by volume of the finished *Standard preparation*) to obtain *Standard preparations*, designated below by letter, having the following concentrations and percentage assignments:

A: (1 in 20); 125 μ g per mL (5.0%).

B: (1 in 33); 75 μ g per mL (3.0%).

C: (1 in 100); 25 μ g per mL (1.0%).

D: (1 in 200); 12.5 μ g per mL (0.5%).

Procedure—Apply separately 20 μ L of the *Test preparation* and 20 μ L of each *Standard preparation* to a suitable thin-

layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Dry the plate with the aid of a stream of cool air. Position the plate in a chromatographic chamber, and develop the chromatograms in *Solvent mixture* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in a stream of cool air. Examine the plate under long-wavelength UV light. Mark the principal and any secondary fluorescent spots. Spray the plate with *Detecting reagent*, and mark the principal and secondary blue spots. Compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations*: the sum of the intensities of secondary spots obtained from the *Test preparation* corresponds to not more than 5.0% of related compounds.

Assay—[NOTE—Conduct this procedure with a minimum exposure to light.]

Mobile phase, Solvent mixture, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay* under *Methylergonovine Maleate*.

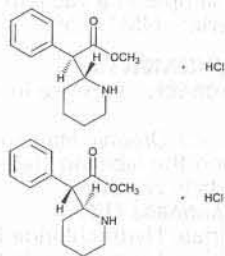
Assay preparation—Place 10 Tablets in 1 500-mL volumetric flask, add 400 mL of *Solvent mixture*, and shake by mechanical means for 15 minutes or until completely disintegrated. Dilute with *Solvent mixture* to volume, and mix. Allow the solution to settle for not less than 30 minutes before use, and then filter to obtain the *Assay preparation*.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of methylergonovine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$) in the portion of Tablets taken by the formula:

$$(L/D)(C)(r_U/r_S)$$

in which *L* is the labeled quantity, in mg, of methylergonovine maleate in each Tablet, *D* is the concentration, in µg per mL, of methylergonovine maleate in the *Assay preparation*, based on the labeled quantity per Tablet and the extent of dilution, *C* is the concentration, in µg per mL, of USP Methylergonovine Maleate RS in the *Standard preparation*, and *r_U* and *r_S* are the responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methylphenidate Hydrochloride



$C_{14}H_{19}NO_2 \cdot HCl$ 269.77
2-Piperidineacetic acid, α-phenyl-, methyl ester, hydrochloride, (*R*,R**)-(±)-;
Methyl α-phenyl-2-piperidineacetate hydrochloride;
(*RS*)-Methyl-2-phenyl-2-[(*RS*)-piperidin-2-yl] acetate, hydrochloride [23655-65-4].

DEFINITION

Methylphenidate Hydrochloride contains NLT 98.0% and NMT 102.0% of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirement for the silver nitrate precipitate test

ASSAY

PROCEDURE

Buffer: 2.7 g/L of monobasic potassium phosphate
Mobile phase: Methanol and *Buffer* (1:2). Adjust with phosphoric acid to a pH of 4.6 ± 0.1 .

System suitability solution: 0.005 mg/mL of USP Methylphenidate Related Compound A RS and 0.5 mg/mL of USP Methylphenidate Hydrochloride RS in the *Mobile phase*

Standard solution: 0.5 mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase*

Sample solution: 0.5 mg/mL of Methylphenidate Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 209 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 µL

Run time: 2 times the retention time of methylphenidate

System suitability

Sample: *System suitability solution*

Suitability requirements

Tailing factor: NMT 3.0 for the methylphenidate peak

Resolution: NLT 2.5 between methylphenidate related compound A and methylphenidate

Relative standard deviation: NMT 2.0% for the methylphenidate peak

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylphenidate Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 ppm • (Official 1-

Jan-2018)

- **ORGANIC IMPURITIES, PROCEDURE 1**

Buffer, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Identify each impurity using the relative retention times in *Table 1*. Calculate the percentage of each impurity

in the portion of Methylphenidate Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity in the *Sample solution*

r_T = sum of the responses for all impurity peaks including the methylphenidate peak in the *Sample solution*

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Erythro isomer ^a	0.58	0.15
Methylphenidate related compound A	0.85	0.5
Methylphenidate	1.0	—
Any individual, unspecified impurity	—	0.10
Total impurities	—	1.0

^a (RS)-Methyl-2-phenyl-2-[(SR)-piperidin-2-yl] acetate.

• ORGANIC IMPURITIES, PROCEDURE 2

[NOTE—Perform this test only if ethylphenidate or bis-1,2-(α -carboxymethylbenzyl) piperidine is a known process impurity.]

Buffer A: 5.7 g of monobasic ammonium phosphate and 1.6 g of 1-octanesulfonate sodium in 1 L of water

Buffer B: Add 4 mL of triethylamine to 1 L of Buffer A. Adjust with phosphoric acid to a pH of 2.9.

Solution A: Acetonitrile and Buffer B (7:43)

Solution B: Acetonitrile and Buffer A (4:1)

System suitability solution: 0.5 mg/mL of USP Methylphenidate Hydrochloride RS; and 3 μ g/mL each of USP Methylphenidate Related Compound A RS, phenylacetic acid, and USP Methylphenidate Hydrochloride Erythro Isomer Solution RS in Solution A

Standard solution: 0.5 μ g/mL of USP Methylphenidate Hydrochloride RS in Solution A

Sample solution: 0.5 mg/mL of Methylphenidate Hydrochloride in Solution A. [NOTE—Allow the solution to stand for at least 2 h.]

Mobile phase: See Table 2. (See also Chromatography (621), System Suitability).

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
7	65	35
10	50	50
12	50	50
13	90	10
16	90	10

[NOTE—Equilibration of the chromatographic system at the initial conditions for a minimum of 30 min is recommended before the first injection.]

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm \times 15-cm; 5- μ m packing L7

Column temperature: 40°

Flow rate: 2.8 mL/min

Injection volume: 10 μ L

System suitability

Sample: System suitability solution. [NOTE—Identify the peaks using the relative retention times in Table 3.]

Suitability requirements

Resolution: NLT 2.7 between methylphenidate related compound A and phenylacetic acid; NLT than 3.6 between phenylacetic acid and erythro isomer

Tailing factor: NMT 2.0 for the methylphenidate peak

Relative standard deviation: NMT 2.0% for the methylphenidate peak; NMT 5.0% for methylphenidate related compound A, phenylacetic acid, and methylphenidate hydrochloride erythro isomer

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of any individual impurity in the portion of Methylphenidate Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity peak from the *Sample solution*

r_S = peak response for the methylphenidate peak from the *Standard solution*

C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylphenidate Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see Table 3)

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Methylphenidate related compound A	0.55	1.1	0.2
Phenylacetic acid	0.67	1.0	0.1
Erythro isomer ^a	0.80	1.0	0.2
Methylphenidate	1.0	—	—
Ethylphenidate ^b	1.22	0.9	0.1
Bis-methylphenidate ^c	1.80	2.6	0.1
Any individual, unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

^a (RS)-Methyl-2-phenyl-2-[(SR)-piperidin-2-yl] acetate.

^b Ethyl 2-phenyl-2-(piperidin-2-yl)acetate.

^c 1,2-Bis(carboxymethylbenzyl)piperidine. [NOTE—Also known as 1,2-(α -carboxymethylbenzyl)piperidine.]

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry a sample in a vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states the procedure with which the article complies.

• USP REFERENCE STANDARDS (11)

USP Methylphenidate Hydrochloride RS

USP Methylphenidate Hydrochloride Erythro Isomer Solution RS

This solution contains 0.5 mg of methylphenidate hydrochloride erythro isomer per mL in methanol.

USP Methylphenidate Related Compound A RS
 α -Phenyl-2-piperidineacetic acid hydrochloride.

$C_{13}H_{17}NO_2 \cdot HCl$ 255.74

Methylphenidate Hydrochloride Tablets

DEFINITION

Methylphenidate Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197M)

Sample: Equivalent to 50 mg of methylphenidate hydrochloride from a portion of powdered Tablets in a 40-mL centrifuge tube. Add 10 mL of chloroform, shake, and centrifuge. Filter the clear extract through a medium-sized sintered-glass funnel into a beaker, and repeat the extraction with an additional 10-mL portion of chloroform. Evaporate the combined chloroform extracts on a steam bath to dryness. Agitate the dried residue with 2 mL of acetonitrile, and filter the mixture through a small sintered-glass funnel. Wash the crystals with an additional 2 mL of acetonitrile, and dry them with the aid of suction.

Acceptance criteria: Meet the requirements

ASSAY

• PROCEDURE

Buffer: Dissolve 1.6 g of anhydrous sodium acetate in 900 mL of water. Adjust with acetic acid to a pH of 4.0. Dilute with water to 1 L.

Mobile phase: Methanol, acetonitrile, and *Buffer* (4:3:3)

Internal standard solution: 0.4 mg/mL of phenylephrine hydrochloride in *Mobile phase*

Standard stock solution: 0.2 mg/mL of USP

Methylphenidate Hydrochloride RS in *Mobile phase*

Standard solution: Mix 10.0 mL of the *Standard stock solution* with 5.0 mL of the *Internal standard solution*

Sample stock solution: 0.2 mg/mL of methylphenidate hydrochloride from finely powdered Tablets (NLT 20 Tablets) prepared as follows. Dissolve in *Mobile phase* using 70% of the final volume. Sonicate for 15 min, and cool to room temperature. Dilute with *Mobile phase* to volume. Pass a portion of this solution through a suitable membrane filter, discarding the first portion of the filtrate. Avoid the use of glass filters. Polypropylene filters are suitable for use.

Sample solution: Mix 10.0 mL of the clear filtrate from the *Sample stock solution* with 5.0 mL of the *Internal standard solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 25-cm; packing L10

Flow rate: 1.5 mL/min

Injection size: 50 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for phenylephrine hydrochloride and methylphenidate hydrochloride are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the analyte and the internal standard peaks

Relative standard deviation: NMT 2.0% from the peak response ratios of the analyte to the internal standard

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

- R_U = peak response ratio of the analyte to the internal standard from the *Sample solution*
 R_S = peak response ratio of the analyte to the internal standard from the *Standard solution*
 C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of Methylphenidate Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• DISSOLUTION (711), Procedure for a Pooled Sample

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Analysis: Determine the amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved by using the procedure in the *Assay*, making any necessary volumetric adjustments.

Tolerances: NLT 75% (Q) of the labeled amount of $C_{14}H_{19}NO_2 \cdot HCl$ is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in tight containers.

• USP REFERENCE STANDARDS (11)

USP Methylphenidate Hydrochloride RS

Methylphenidate Hydrochloride Extended-Release Tablets

DEFINITION

Methylphenidate Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$).

IDENTIFICATION

• A. INFRARED ABSORPTION

Sample: Place a portion of powdered Tablets, equivalent to 100 mg of methylphenidate hydrochloride, in a 100-mL beaker. Add 20 mL of chloroform, stir for 5 min, and filter, collecting the filtrate. Evaporate the filtrate to about 5 mL. Add ethyl ether slowly, with stirring, until crystals form. Filter the crystals, wash with ethyl ether, and dry at 80° for 30 min.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the crystals so obtained exhibits maxima only at the same wavelengths as those of a similar preparation of USP Methylphenidate Hydrochloride RS.

• B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Mobile phase: Dissolve 2 g of octanesulfonic acid sodium salt in 730 mL of water. Adjust with phosphoric acid to a pH of 2.7. Mix with 270 mL of acetonitrile.

Solution A: Acidified water; adjusted with phosphoric acid to a pH of 3

Diluent A: Acetonitrile and *Solution A* (25:75)

Diluent B: Acetonitrile and methanol (50:50)

System suitability solution: 80 μ g/mL of USP

Methylphenidate Hydrochloride RS, 1 μ g/mL of

methylphenidate hydrochloride erythro isomer from

USP Methylphenidate Hydrochloride Erythro Isomer So-

lution RS, and 2 µg/mL of USP Methylphenidate Related Compound A RS in *Diluent A*

Standard solution: 0.1 mg/mL of USP Methylphenidate Hydrochloride RS in *Diluent A*

Sample stock solution: Nominally 1 mg/mL of methylphenidate hydrochloride prepared as follows. Dissolve NLT 10 Tablets in a suitable volumetric flask with 20% of the total flask volume of *Diluent B*. [NOTE—Alternatively, a portion of powder from NLT 10 Tablets may be transferred to a suitable volumetric flask and suspended in 20% of the total flask volume of *Diluent B*.] Stir for 4 h. Dilute with *Solution A* to volume.

Sample solution: Nominally 0.1 mg/mL of methylphenidate hydrochloride in *Solution A* from the *Sample stock solution*. [NOTE—Centrifuge before chromatographic analysis.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 25 µL

Run time: 2 times the retention time of methylphenidate

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 6* for relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between methylphenidate related compound A and methylphenidate hydrochloride erythro isomer; NLT 6.0 between the methylphenidate and erythro isomer peaks, *System suitability solution*

Tailing factor: NMT 2.0 for the methylphenidate peak, *Standard solution*

Relative standard deviation: NMT 2.0% for the methylphenidate peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Times: 1, 2, 3.5, 5, and 7 h

Buffer: Dissolve 1.6 g of anhydrous sodium acetate in 900 mL of water. Adjust with acetic acid to a pH of 4.0 and dilute with water to 1000 mL.

Mobile phase: Methanol, acetonitrile, and *Buffer* (40:30:30)

Internal standard solution: 0.4 mg/mL of phenylephrine hydrochloride in *Mobile phase*

Standard stock solution: $(1.5 \times [L/500])$ mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase* where L is the label claim of methylphenidate hydrochloride in mg/Tablet

Standard solution: Transfer 10.0 mL of the *Standard stock solution* to a glass-stoppered, 25-mL conical flask, add 5.0 mL of the *Internal standard solution*, and mix.

Sample stock solution: Use portions of the solution under test passed through a suitable filter of 0.45-µm pore size. Do not use glass fiber filters.

Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a glass-stoppered, 25-mL conical flask, add 5.0 mL of the *Internal standard solution*, and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; packing L10

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for phenylephrine hydrochloride and methylphenidate hydrochloride are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the analyte and internal standard peaks

Relative standard deviation: NMT 2.0% for the peak response ratios of the analyte to the internal standard

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved by using the procedure in the *Assay*, making any necessary volumetric adjustments.

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
1	25–45
2	40–65
3.5	55–80
5	70–90
7	NLT 80

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

For products labeled for dosing every 24 h

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Acidified water; adjusted with phosphoric acid to a pH of 3; 50 mL at $37 \pm 0.5^\circ$

Apparatus 7 (see *Drug Release* (724)): 30 cycles/min; 2–3 cm amplitude. Follow *Sample preparation A* using a metal spring sample holder (*Figure 5d*). Place one Tablet in the holder with the Tablet orifice facing down, and cover the top of the holder with Parafilm™. At the end of each specified test interval, the systems are transferred to the next row of new test tubes containing 50 mL of fresh *Medium*.

Times: 1-h intervals for a duration of 10 h

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved by using the following method.

Solution A: Dissolve 2.0 g of sodium 1-octanesulfonate in 700 mL of water, mix well, and adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Solution A* (30:70)

Diluent: Acetonitrile and *Medium* (25:75)

Standard stock solution: 0.3 mg/mL of USP Methylphenidate Hydrochloride RS in *Diluent*

Standard solutions: Prepare at least six solutions by making serial dilutions of the *Standard stock solution* in *Diluent* to bracket the expected drug concentration range.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.2-mm × 5-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 25 μL

System suitability

Sample: Middle range concentration of the *Standard solutions*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2% for the peak response of the analyte; NMT 2% for the retention time of the analyte

Analysis

Samples: *Standard solutions* and the solution under test

Construct a calibration curve by plotting the peak response versus the concentration of the *Standard solutions*. Determine the amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in each interval by linear regression analysis of the standard curve.

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
1	12–32
4	40–60
10	NLT 85
3–6 (avg)	9–15 (/h)

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Calculate the average percentage released from 3–6 h:

$$\text{Result} = (Y - X)/3$$

Y = cumulative drug released from 0–6 h

X = cumulative drug released from 0–3 h

For products labeled for dosing every 24 h

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: pH 6.8 phosphate buffer (6.8 g/L of monobasic potassium phosphate in water; adjusted with 2 N sodium hydroxide or 10% phosphoric acid to a pH of 6.80); 900 mL

Apparatus 1: 100 rpm

Times: 0.75, 4, and 10 h

Buffer: pH 4.0 phosphate buffer (2.72 g/L of monobasic potassium phosphate in water; adjusted with 2 N sodium hydroxide or 10% phosphoric acid to a pH of 4.00)

Mobile phase: Acetonitrile and *Buffer* (17.5: 82.5)

Standard solution: 0.06 mg/mL of USP Methylphenidate Hydrochloride RS in 0.1 N hydrochloric acid

Sample solution: Pass a portion of the solution under test through a suitable polytetrafluoroethylene (PTFE) filter of 0.45-μm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.0-mm × 5-cm; 2.5-μm packing L1

Column temperature: 50°

Flow rate: See *Table 3*.

Table 3

Time (min)	Flow Rate (mL/min)
0.0	0.75
2.5	0.75
3.0	2.00
6.0	2.00
6.5	0.75
7.0	0.75

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for methylphenidate related compound A, the erythro isomer, and methylphenidate are 0.47, 0.65, and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i) shown in *Table 4*:

$$\text{Result}_i = (r_u/r_s) \times C_s$$

r_u = sum of the peak responses of methylphenidate and methylphenidate related compound A from the *Sample solution*

r_s = peak response of methylphenidate from the *Standard solution*

C_s = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution*

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at each time point (i) shown in *Table 4*:

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_s)] + [C_1 \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_s)]] + [(C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

C_i = concentration of methylphenidate hydrochloride in the portion of sample withdrawn at time point (i) (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_s = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 4*.

Table 4

Time Point (i)	Time (h)	Amount Dissolved (%)
1	0.75	12–30
2	4	55–80
3	10	NLT 80

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: 0.001 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Times: 1, 2, 6, and 10 h

Mobile phase: Acetonitrile and water (20:80). For every L of *Mobile phase* add 1.0 mL of formic acid and 0.2 mL of trifluoroacetic acid.

Standard solution: 0.02 mg/mL of USP Methylphenidate Hydrochloride RS in *Mobile phase*

Sample solution: Pass a portion of the solution under test through a suitable PTFE filter of 0.45- μ m pore size. Do not use glass fiber filters.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.0-mm \times 15-cm; 3- μ m packing L1

Column temperature: 40°

Flow rate: 0.75 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the concentration (C_i) of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i) shown in *Table 5*:

$$Result_i = (r_u/r_s) \times C_s$$

r_u = peak response of methylphenidate from the *Sample solution*

r_s = peak response of methylphenidate from the *Standard solution*

C_s = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution*

Calculate the percentage of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at each time point (i) shown in *Table 5*:

$$Result_1 = C_i \times V \times (1/L) \times 100$$

$$Result_2 = \{[C_2 \times (V - V_s)] + [C_i \times V_s]\} \times (1/L) \times 100$$

$$Result_3 = \{[C_3 \times [V - (2 \times V_s)]] + [(C_2 + C_i) \times V_s]\} \times (1/L) \times 100$$

$$Result_4 = \{[C_4 \times [V - (3 \times V_s)]] + [(C_3 + C_2 + C_i) \times V_s]\} \times (1/L) \times 100$$

C_i = concentration of methylphenidate hydrochloride in the portion of sample withdrawn at time point (i) (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

V_s = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 5*.

Table 5

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	20–40
2	2	35–55
3	6	65–85
4	10	NLT 80

The percentages of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase: Dissolve 2 g of sodium-1-octanesulfonate in 730 mL of water. Adjust with phosphoric acid to a pH of 2.7. Mix with 270 mL of acetonitrile.

Solution A: Acidified water; adjusted with phosphoric acid to a pH of 3

Diluent A: Acetonitrile and *Solution A* (25:75)

Diluent B: Acetonitrile and methanol (50:50)

System suitability solution: 80 μ g/mL of USP

Methylphenidate Hydrochloride RS, 1 μ g/mL of methylphenidate hydrochloride erythro isomer from USP Methylphenidate Hydrochloride Erythro Isomer Solution RS, and 2 μ g/mL of USP Methylphenidate Related Compound A RS in *Diluent A*

Standard solution: 0.2 μ g/mL of USP Methylphenidate Hydrochloride RS, 0.5 μ g/mL of methylphenidate hydrochloride erythro isomer from USP Methylphenidate Hydrochloride Erythro Isomer Solution RS, and 1.5 μ g/mL of USP Methylphenidate Related Compound A RS in *Diluent A*

Sample stock solution: Nominally 1 mg/mL of methylphenidate hydrochloride prepared as follows. Dissolve NLT 10 Tablets in a suitable volumetric flask with 20% of the total flask volume of *Diluent B*. [NOTE—Alternatively, a portion of powder from NLT 10 Tablets may be transferred to a suitable volumetric flask and suspended in 20% of the total flask volume of *Diluent B*.] Stir for 4 h. Dilute with *Solution A* to volume.

Sample solution: 0.1 mg/mL of methylphenidate hydrochloride in *Solution A* from the *Sample stock solution*. [NOTE—Centrifuge before chromatographic analysis.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm \times 15-cm; 5- μ m packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 25 μ L

Run time: 2 times the retention time of methylphenidate

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 6.0 between the methylphenidate and erythro isomer peaks

Tailing factor: NMT 2.0 for the methylphenidate peak

Relative standard deviation: NMT 2.0% for the methylphenidate peak; NMT 4.0% each for the methylphenidate related compound A and erythro isomer peaks

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of methylphenidate related compound A or erythro isomer in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of methylphenidate related compound A or erythro isomer from the *Sample solution*
 r_S = peak response of methylphenidate related compound A or erythro isomer from the *Standard solution*
 C_S = concentration of USP Methylphenidate Related Compound A RS or methylphenidate hydrochloride erythro isomer in the *Standard solution* (mg/mL)
 C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of each unspecified degradation product from the *Sample solution*
 r_S = peak response of USP Methylphenidate Hydrochloride RS from the *Standard solution*
 C_S = concentration of USP Methylphenidate Hydrochloride RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of methylphenidate hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 6.

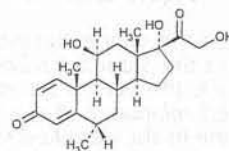
Table 6

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methylphenidate related compound A	0.47	1.5
Erythro isomer ^a	0.65	0.5
Methylphenidate	1.0	—
Any unspecified degradation product	—	0.2
Total degradation products	—	2.5

^a Methyl (RS,SR)-2-phenyl-2-(piperidin-2-yl) acetate.

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- LABELING:** The labeling states the *Dissolution* test with which the product complies if other than *Test 1*.
- USP REFERENCE STANDARDS (11)**
 - USP Methylphenidate Hydrochloride RS
 - USP Methylphenidate Hydrochloride Erythro Isomer Solution RS
 - This solution contains 0.5 mg of methylphenidate hydrochloride erythro isomer per mL in methanol.
 - USP Methylphenidate Related Compound A RS
 - α -Phenyl-2-piperidineacetic acid hydrochloride.
 - $C_{13}H_{17}NO_2 \cdot HCl$ 255.74

Methylprednisolone

$C_{22}H_{30}O_5$ 374.47

Pregna-1,4-diene-3,20-dione, 11,17,21-trihydroxy-6-methyl-, (6 α ,11 β)-, 11 β ,17,21-Trihydroxy-6 α -methylpregna-1,4-diene-3,20-dione [83-43-2].

» Methylprednisolone contains not less than 97.0 percent and not more than 103.0 percent of $C_{22}H_{30}O_5$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—
USP Methylprednisolone RS

Identification

A: *Infrared Absorption* (197K).

B: *Ultraviolet Absorption* (197U)—

Solution: 10 μ g per mL.

Medium: alcohol.

Absorptivities at 243 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: Dissolve about 5 mg in 2 mL of sulfuric acid: a red color is produced.

Specific rotation (781S): between +79° and +86°.

Test solution: 5 mg per mL, in dioxane.

Loss on drying (731)—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.2%.

Chromatographic purity

Mobile phase—Prepare a filtered and degassed mixture of water, tetrahydrofuran, dimethyl sulfoxide, and butanol (149:40:10:1). Make adjustments if necessary (see *System Suitability in Chromatography* (621)).

Diluting solution—Prepare a filtered mixture of water, tetrahydrofuran, and glacial acetic acid (72:25:3).

Standard solution—Dissolve an accurately weighed quantity of USP Methylprednisolone RS in *Diluting solution*. Dilute quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 0.01 mg per mL.

Test solution—Transfer about 25 mg of Methylprednisolone, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Diluting solution* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm \times 20-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 800 theoretical plates; and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the

peak responses. Calculate the percentage of each impurity in the portion of Methylprednisolone taken by the formula:

$$100(C_s / C_u)(r_i / r_s)$$

in which C_s and C_u are the concentrations, in mg per mL, of Methylprednisolone in the *Standard solution* and the *Test solution*, respectively; r_i is the peak response for each impurity obtained from the *Test solution*; and r_s is the peak response for methylprednisolone in the *Standard solution*: not more than 1.0% of any individual impurity is found, and not more than 2.0% of total impurities is found.

Assay—

Mobile phase—Prepare a solution containing a mixture of butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30).

Internal standard solution—Dissolve prednisone in a 3 in 100 solution of glacial acetic acid in chloroform to obtain a solution having a concentration of about 0.2 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Methylprednisolone RS in *Internal standard solution* to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Using about 10 mg of Methylprednisolone, accurately weighed, proceed as directed for *Standard preparation*.

Chromatographic system (see *Chromatography*)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 25-cm column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R , between the methylprednisolone and internal standard peaks is not less than 4.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks: the relative retention times are about 0.7 for prednisone and 1.0 for methylprednisolone. Calculate the quantity, in percent, of $C_{22}H_{30}O_5$ in the portion of Methylprednisolone taken by the formula:

$$100(C_s / C_u)(R_u / R_s)$$

in which C_s is the concentration of methylprednisolone, in mg per mL, in the *Standard preparation*; C_u is the nominal concentration, in mg per mL, of Methylprednisolone in the *Assay preparation*; and R_u and R_s are the ratios of the peak responses for the methylprednisolone peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Methylprednisolone Tablets

» Methylprednisolone Tablets contain not less than 92.5 percent and not more than 107.5 percent of the labeled amount of methylprednisolone ($C_{22}H_{30}O_5$).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Methylprednisolone RS

Identification—Powder a number of Tablets, equivalent to about 40 mg of methylprednisolone, and digest with 25 mL of solvent hexane for 15 minutes. Filter, and discard the fil-

trate. Digest the residue with 25 mL of chloroform for 15 minutes. Filter, evaporate the filtrate to dryness, and dry at 105° for 2 hours: the residue so obtained responds to *Identification tests A and C* under *Methylprednisolone*.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Measure the UV absorption of filtered aliquots removed from the *Dissolution Medium* and suitably diluted, if necessary, in 1-cm cells at 246 nm, with a suitable spectrophotometer, using water as the blank and utilizing a standard curve, representing the absorbance versus concentration of USP Methylprednisolone RS. [NOTE—Dissolve about 20 mg of USP Methylprednisolone RS, accurately weighed, in 1 mL of alcohol, dilute in a 1000-mL volumetric flask with water to volume, and mix. Prepare quantitative dilutions of this solution for the development of a standard curve.]

Tolerances—Not less than 70% (Q) of the labeled amount of $C_{22}H_{30}O_5$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

PROCEDURE FOR CONTENT UNIFORMITY—

Mobile phase, *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the Assay under *Methylprednisolone*.

Test preparation—Place 1 Tablet in a suitable container. For tablet labeled strengths of 10 mg or less, add 0.5 mL of water. For tablet labeled strengths greater than 10 mg, add 1.0 mL of water. Allow the tablet to stand for about 2 minutes, then swirl the container to disperse the tablet. Add 5.0 mL of *Internal standard solution* for each mg of labeled tablet strength, shake for 15 minutes, and filter or centrifuge a portion of the test specimen. Analyze the clear solution as directed under *Procedure*.

Procedure—Proceed as directed for *Procedure* in the Assay under *Methylprednisolone*. Calculate the quantity, in mg, of $C_{22}H_{30}O_5$ in the Tablet taken by the formula:

$$(FW_s)(R_u / R_s)$$

in which F is the ratio of the volume of *Internal standard preparation*, in mL, in the *Test preparation* to the volume, in mL, of the *Internal standard preparation* in the *Standard preparation*; W_s is the weight, in mg, of USP Methylprednisolone RS taken for the *Standard preparation*; and the other terms are as defined for *Procedure* in the Assay under *Methylprednisolone*.

Assay—

Mobile phase, *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the Assay under *Methylprednisolone*.

Assay preparation—Accurately weigh 20 Tablets, and grind to a fine powder in a mortar and pestle. Accurately weigh a portion of the powder, equivalent to about 10 mg of methylprednisolone, and transfer to a suitable container. Add 2.5 mL of water to the ground tablet material and swirl to form a fine slurry. Add 50.0 mL of *Internal standard solution*, and shake for 15 minutes. Filter or centrifuge a portion of the liquid so obtained, if necessary, and analyze the clear solution as directed under *Procedure*.

Procedure—Proceed as directed for *Procedure* in the Assay under *Methylprednisolone*. Calculate the quantity, in mg, of $C_{22}H_{30}O_5$ in the portion of Tablets taken by the formula:

$$50C(R_u / R_s)$$

in which the terms are as defined therein.

Methylprednisolone Acetate

$C_{24}H_{32}O_6$ 416.51
 Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-11,17-dihydroxy-6-methyl-, (6 α ,11 β)-;
 11 β ,17,21-Trihydroxy-6 α -methylpregna-1,4-diene-3,20-dione 21-acetate [53-36-1].

DEFINITION

Methylprednisolone Acetate contains NLT 97.0% and NMT 103.0% of methylprednisolone acetate ($C_{24}H_{32}O_6$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)
 - Analytical wavelength: 243 nm
 - Standard solution: 10 μ g/mL of USP Methylprednisolone Acetate RS in alcohol
 - Sample solution: 10 μ g/mL of methylprednisolone acetate in alcohol
 - Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY

- **PROCEDURE**
 - Mobile phase: *n*-Butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)
 - Internal standard solution: 6 mg/mL of prednisone prepared as follows. Transfer an appropriate amount of prednisone to a suitable volumetric flask. Add 3% of the flask volume of glacial acetic acid, and sonicate. Dilute with chloroform to volume, slowly adding the chloroform. Sonicate, and shake to dissolve.
 - Standard solution: 0.2 mg/mL of USP Methylprednisolone Acetate RS prepared as follows. Transfer an appropriate amount of USP Methylprednisolone Acetate RS to a suitable volumetric flask, and add 5% of the flask volume of the *Internal standard solution*. Dilute with chloroform to volume, and shake to dissolve.
 - Sample solution: 0.2 mg/mL of Methylprednisolone Acetate prepared as follows. Transfer an appropriate amount of methylprednisolone acetate to a suitable volumetric flask, and add 5% of the flask volume of the *Internal standard solution*. Dilute with chloroform to volume, and shake to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm \times 25-cm; packing L3

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for methylprednisolone acetate and prednisone are about 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 2.5 between methylprednisolone acetate and prednisone

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
 Calculate the percentage of methylprednisolone acetate ($C_{24}H_{32}O_6$) in the portion of Methylprednisolone Acetate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak height ratio of methylprednisolone acetate to prednisone from the *Sample solution*

R_S = peak height ratio of methylprednisolone acetate to prednisone from the *Standard solution*

C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylprednisolone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

ORGANIC IMPURITIES

Mobile phase: Tetrahydrofuran and water (51:149)

Diluent: Tetrahydrofuran, acetonitrile, glacial acetic acid, and water (250:250:1:499)

Standard solution: 20 μ g/mL of USP Methylprednisolone Acetate RS in *Diluent*. Sonicate, if necessary, to dissolve.

Sample solution: 1 mg/mL of Methylprednisolone Acetate in *Diluent*. Sonicate, if necessary, to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Methylprednisolone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response for methylprednisolone acetate from the *Standard solution*

C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylprednisolone Acetate in the *Sample solution* (mg/mL)

Acceptance criteria

Any individual impurity: NMT 1.0%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation** (781S)

Sample solution: 10 mg/mL in dioxane

Acceptance criteria: +97° to +105°

- **LOSS ON DRYING** (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

- **USP REFERENCE STANDARDS** (11)

USP Methylprednisolone Acetate RS

Methylprednisolone Acetate Cream

DEFINITION

Methylprednisolone Acetate Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of methylprednisolone acetate ($C_{24}H_{32}O_6$).

IDENTIFICATION**• A.**

Analysis: Use the thin-layer chromatogram, prepared as directed in *Analysis 1* in the *Assay*.

Acceptance criteria: The R_f value of the principal spot from the *Sample stock solution* corresponds to that from the *Standard stock solution*.

ASSAY**• PROCEDURE**

(See *Chromatography* (621).)

Solution A: Alcohol and chloroform (1:1)

Solution B: Alcohol and tetramethylammonium hydroxide TS (9:1)

Standard stock solution: 500 µg/mL of USP Methylprednisolone Acetate RS in *Solution A*

Sample stock solution: Transfer the equivalent of 5 mg of methylprednisolone acetate from Cream to a 125-mL separator, and add 50 mL of solvent hexane. Extract with three 10-mL portions of acetonitrile, and evaporate the combined extracts on a steam bath with the aid of a current of air nearly to dryness. Transfer the residue to a 10-mL volumetric flask with the aid of one 5-mL portion and two 2-mL portions of *Solution A*, and dilute with *Solution A* to volume.

Adsorbent: 0.5-mm layer of chromatographic silica gel mixture

Application volume: 250 µL

Developing solvent system: Ethyl acetate and chloroform (7:5)

Analysis 1

Samples: *Standard stock solution* and *Sample stock solution*

Divide the plate into three equal sections, with the left and right sections to be used for the *Sample stock solution* and *Standard stock solution*, respectively, and the center section for the blank. Apply the solutions as streaks 2.5 cm from the bottom of the designated section of the plate, and dry the streaks with the aid of a current of air. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Locate the principal bands from the *Standard stock solution* and the *Sample stock solution* (see also *Identification test A*) by viewing under short-wavelength UV light.

Standard solution, Sample solution, and Blank: Mark the *Standard stock solution* and *Sample stock solution* bands and the corresponding band section for the *Blank* on the TLC plate from *Analysis 1*. Quantitatively remove the silica gel containing these bands, and transfer to separate glass-stoppered, 50-mL centrifuge tubes. Add 25.0 mL of alcohol to each tube, shake for 2 min, and centrifuge for 5 min. Transfer 20.0 mL of each supernatant to separate glass-stoppered, 50-mL conical flasks. Add 2.0 mL of blue tetrazolium TS to each solution, mix, and add 2.0 mL of *Solution B* to each flask. Mix, and allow the solutions to stand in the dark for 90 min.

Instrumental conditions

Mode: Vis

Analytical wavelength: Maximum absorbance at about 525 nm

Cell: 1 cm

Analysis 2

Samples: *Standard solution*, *Sample solution*, and *Blank*
Determine the absorbances of the *Standard solution* and the *Sample solution* at the wavelength of maximum absorbance against the *Blank*.

Calculate the percentage of the labeled amount of methylprednisolone acetate ($C_{24}H_{32}O_6$) in the portion of Cream taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard stock solution* (µg/mL)

C_U = nominal concentration of methylprednisolone in the *Sample stock solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- MINIMUM FILL (755):** Meets the requirements

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers, protected from light.
- USP REFERENCE STANDARDS (11)**
USP Methylprednisolone Acetate RS

Methylprednisolone Acetate Injectable Suspension

DEFINITION

Methylprednisolone Acetate Injectable Suspension is a sterile suspension of Methylprednisolone Acetate in a suitable aqueous medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of methylprednisolone acetate ($C_{24}H_{32}O_6$).

IDENTIFICATION**• A. INFRARED ABSORPTION (197K)**

Sample: Nominally 100 mg of methylprednisolone acetate from Injectable Suspension

Analysis: Filter the *Sample* through paper. Wash the residue with several 5-mL portions of water, and dry at 105° for 3 h.

Acceptance criteria: Meets the requirements

ASSAY**• PROCEDURE**

Mobile phase: *n*-Butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)

Internal standard solution: 6 mg/mL of prednisone prepared as follows. Transfer an appropriate amount of prednisone to a suitable volumetric flask. Add 3% of the flask volume of glacial acetic acid, and sonicate. Dilute with chloroform to volume, slowly adding the chloroform. Sonicate, and shake to dissolve.

Standard solution: 0.2 mg/mL of USP Methylprednisolone Acetate RS prepared as follows. Transfer an appropriate amount of USP Methylprednisolone Acetate RS to a suitable volumetric flask, and add 5% of the flask volume of the *Internal standard solution*. Dilute with chloroform to volume, and shake to dissolve.

Sample solution: Swirl Injectable Suspension to ensure uniformity before analysis. Transfer a suitable quantity of Injectable Suspension equivalent to 40 mg of methylprednisolone acetate to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, dilute with chloroform to volume, and shake for 15 min or until the aqueous layer is clear. Transfer 4.0 mL of the chloroform layer to a suitable vial, add 30 mL of chloroform and a small quantity (about 400 mg) of anhydrous sodium sulfate, shake for 5 min. Use the clear solution.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4-mm × 25-cm; packing L3**Flow rate:** 1 mL/min**Injection volume:** 10 µL**System suitability****Sample:** *Standard solution*

[NOTE—The relative retention times for methylprednisolone acetate and prednisone are 1.0 and 1.3, respectively.]

Suitability requirements**Resolution:** NLT 2.5 between methylprednisolone acetate and prednisone**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of methylprednisolone acetate (C₂₄H₃₂O₆) in the portion of Injectable Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

 R_U = peak height ratio of methylprednisolone acetate to prednisone from the *Sample solution* R_S = peak height ratio of methylprednisolone acetate to prednisone from the *Standard solution* C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (mg/mL) C_U = nominal concentration of methylprednisolone acetate in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

SPECIFIC TESTS

- **pH** (791): 3.0–7.0
- **PARTICLE SIZE**

Analysis: Transfer 1 drop to a microscope slide, and spread it evenly, diluting with water if necessary, to decrease the density of the field. Examine the slide under a microscope equipped with a calibrated ocular micrometer, using 400× magnification. Scan the entire slide, and note the size of the individual particles.

Acceptance criteria: NLT 99% of the particles are less than 20 µm in length when measured along the longest axis, and NLT 75% of the particles are less than 10 µm.

- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS** (11)
USP Methylprednisolone Acetate RS

Methylprednisolone Hemisuccinate

C₂₆H₃₄O₈ 474.54
 Pregna-1,4-diene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11,17-dihydroxy-6-methyl-, (6α,11β)-;
 11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione 21-(hydrogen succinate) [2921-57-5].

DEFINITION

Methylprednisolone Hemisuccinate contains NLT 97.0% and NMT 103.0% of methylprednisolone hemisuccinate (C₂₆H₃₄O₈), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)

- **B. ULTRAVIOLET ABSORPTION** (197U)

Standard solution: 20 µg/mL of USP Methylprednisolone Hemisuccinate RS in alcohol**Sample solution:** 20 µg/mL of methylprednisolone hemisuccinate in alcohol**Analytical wavelength:** 243 nm**Acceptance criteria:** Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.**ASSAY****PROCEDURE****Solution A:** Chloroform and glacial acetic acid (97:3)**Mobile phase:** Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)**Internal standard solution:** 6 mg/mL of USP Fluorometholone RS in tetrahydrofuran

Standard solution: 0.4 mg/mL of USP Methylprednisolone Hemisuccinate RS prepared as follows. Transfer a suitable quantity of USP Methylprednisolone Hemisuccinate RS to a suitable volumetric flask, and add 5.0% of the flask volume of *Internal standard solution*. Dilute with *Solution A* to volume.

Sample solution: 0.4 mg/mL of Methylprednisolone Hemisuccinate prepared as follows. Transfer a suitable quantity of Methylprednisolone Hemisuccinate to a suitable volumetric flask, and add 5.0% of the flask volume of *Internal standard solution*. Dilute with *Solution A* to volume.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4-mm × 30-cm; packing L3**Injection volume:** 4–8 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 2.0 between methylprednisolone hemisuccinate and fluorometholone**Relative standard deviation:** NMT 2.0% for six replicate injections**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of methylprednisolone hemisuccinate (C₂₆H₃₄O₈) in the portion of Methylprednisolone Hemisuccinate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

 R_U = peak area ratio of methylprednisolone hemisuccinate to fluorometholone from the *Sample solution* R_S = peak area ratio of methylprednisolone hemisuccinate to fluorometholone from the *Standard solution* C_S = concentration of USP Methylprednisolone Hemisuccinate RS in the *Standard solution* (mg/mL) C_U = concentration of Methylprednisolone Hemisuccinate in the *Sample solution* (mg/mL)**Acceptance criteria:** 97.0%–103.0% on the dried basis**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.2%

- **ORGANIC IMPURITIES**

Mobile phase: Tetrahydrofuran, formic acid, and water (255:1:745)

Diluent: Tetrahydrofuran, acetonitrile, acetic acid, and water (25:25:3:47)

Standard solution: 0.02 mg/mL of USP Methylprednisolone Hemisuccinate RS in *Diluent*

Sample solution: 1 mg/mL of Methylprednisolone Hemisuccinate in *Diluent*. Shake or sonicate to aid in solubilization.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 20-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 5000 theoretical plates

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Methylprednisolone Hemisuccinate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of methylprednisolone hemisuccinate from the *Standard solution*

C_S = concentration of USP Methylprednisolone Hemisuccinate RS in the *Standard solution* (mg/mL)

C_U = concentration of Methylprednisolone Hemisuccinate in the *Sample solution* (mg/mL)

Acceptance criteria

Any individual impurity: NMT 1.0%

Total impurities: NMT 2.0%

SPECIFIC TESTS

• OPTICAL ROTATION, *Specific Rotation* (781S)

Sample solution: 10 mg/mL of Methylprednisolone Hemisuccinate in dioxane

Acceptance criteria: +87° to +95°

• LOSS ON DRYING (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in tight containers.

• USP REFERENCE STANDARDS (11)

USP Fluorometholone RS

USP Methylprednisolone Hemisuccinate RS

Methylprednisolone Sodium Succinate

$C_{26}H_{33}NaO_8$ 496.53

Pregna-1,4-diene-3,20-dione, 21-(3-carboxy-1-oxopropoxy)-11,17-dihydroxy-6-methyl-, monosodium salt, (6α,11β)-; 11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione 21-(sodium succinate) [2375-03-3].

DEFINITION

Methylprednisolone Sodium Succinate contains NLT 97.0% and NMT 103.0% of methylprednisolone sodium succinate ($C_{26}H_{33}NaO_8$), calculated on the dried basis.

IDENTIFICATION

• A. INFRARED ABSORPTION

Sample: 100 mg of Methylprednisolone Sodium Succinate

Analysis: Transfer the *Sample* to a separator, dissolve in 10 mL of water, add 1 mL of 3 N hydrochloric acid, and extract immediately with 50 mL of chloroform. Filter the chloroform extract through cotton, evaporate on a steam bath to dryness, and dry under vacuum at 60° for 3 h.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the residue so obtained exhibits maxima only at the same wavelengths as those of a similar preparation of USP Methylprednisolone Hemisuccinate RS.

• B. ULTRAVIOLET ABSORPTION (197U)

Standard solution: 20 μg/mL of USP Methylprednisolone Hemisuccinate RS in methanol

Sample solution: 20 μg/mL of Methylprednisolone Sodium Succinate in methanol

Analytical wavelength: 243 nm

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

• C. The sample imparts an intense yellow color to a nonluminous flame.

ASSAY

• PROCEDURE

Solution A: 5 mg/mL of blue tetrazolium in alcohol

Solution B: Alcohol and tetramethylammonium hydroxide TS (9:1)

Standard solution: Proceed as directed for *Assay for Steroids* (351), *Standard Preparation*, preparing 12.5 μg/mL of USP Methylprednisolone Hemisuccinate RS in alcohol.

Sample solution: 12.5 μg/mL of Methylprednisolone Sodium Succinate in alcohol

Blank: Alcohol

Instrumental conditions

Mode: Vis

Analytical wavelength: 525 nm

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*
Pipet 20.0 mL of the *Blank*, *Standard solution*, and *Sample solution* into three different glass-stoppered, 50-mL conical flasks. Add 2.0 mL of *Solution A*, and mix. To each flask add 4.0 mL of *Solution B*. Mix, and allow to stand in the dark for 90 min. Add 1.0 mL of glacial acetic acid, and mix. Without delay, determine the absorbances of the *Standard solution* and the *Sample solution* against the *Blank*.

Calculate the percentage of methylprednisolone sodium succinate ($C_{26}H_{33}NaO_8$) in the portion of Methylprednisolone Sodium Succinate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Methylprednisolone Hemisuccinate RS in the *Standard solution* (μg/mL)

C_U = concentration of Methylprednisolone Sodium Succinate in the *Sample solution* (μg/mL)

M_{r1} = molecular weight of methylprednisolone sodium succinate, 496.53

M_{r2} = molecular weight of methylprednisolone hemisuccinate, 474.54

Acceptance criteria: 97.0%–103.0% on the dried basis

OTHER COMPONENTS

• SODIUM CONTENT

Sample solution: Dissolve, with gentle heating, about 1 g of Methylprednisolone Sodium Succinate in 75 mL of glacial acetic acid. Add 20 mL of dioxane, then add 1 drop of crystal violet TS.

Titrimetric system

Mode: Direct

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: Titrate with *Titrant* to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 2.299 mg of sodium (Na).

Acceptance criteria: 4.49%–4.77% on the dried basis

SPECIFIC TESTS• **OPTICAL ROTATION (781S), Specific Rotation**

Sample solution: 10 mg/mL of Methylprednisolone Sodium Succinate in alcohol

Acceptance criteria: +96° to +104°

• **LOSS ON DRYING (731)**

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 3.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP Methylprednisolone Hemisuccinate RS

Methylprednisolone Sodium Succinate for Injection

DEFINITION

Methylprednisolone Sodium Succinate for Injection is a sterile mixture of Methylprednisolone Sodium Succinate with suitable buffers. It may be prepared from Methylprednisolone Sodium Succinate or from Methylprednisolone Hemisuccinate with the aid of Sodium Hydroxide or Sodium Carbonate. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of methylprednisolone ($C_{22}H_{30}O_5$) in the volume of constituted solution designated on the label.

IDENTIFICATION• **A. INFRARED ABSORPTION**

Sample: Nominally 100 mg of methylprednisolone sodium succinate from Methylprednisolone Sodium Succinate for Injection

Analysis: Transfer the *Sample* to a separator, dissolve in 10 mL of water, add 1 mL of 3 N hydrochloric acid, and extract immediately with 50 mL of chloroform. Filter the chloroform extract through cotton, evaporate on a steam bath to dryness, and dry under vacuum at 60° for 3 h.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the residue so obtained exhibits maxima only at the same wavelengths as those of a similar preparation of USP Methylprednisolone Hemisuccinate RS.

ASSAY• **PROCEDURE**

Diluent: Chloroform and glacial acetic acid (97:3)

Mobile phase: Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)

Standard stock solution: 0.30 mg/mL of USP Methylprednisolone RS in *Diluent*

Internal standard solution: 3 mg/mL of USP Fluorometholone RS in tetrahydrofuran

Standard solution: 0.65 mg/mL of USP Methylprednisolone Hemisuccinate RS prepared as follows.

Transfer an appropriate amount of USP Methylprednisolone Hemisuccinate RS to a suitable volumetric flask. Pipet 10% of the flask volume of the *Internal stan-*

ard solution and 10% of the flask volume of the *Standard stock solution*. Dilute with *Diluent* to volume.

Sample solution: Transfer a suitable quantity of constituted solutions (mix the constituted solutions prepared from the contents of 10 vials of Methylprednisolone Sodium Succinate for Injection) equivalent to 50 mg of methylprednisolone to a suitable flask containing 10.0 mL of the *Internal standard solution*, and dilute with *Diluent* to 100.0 mL. Shake thoroughly for 5 min, then allow the phases to separate, discarding the upper phase.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L3

Flow rate: 1 mL/min

Injection volume: 6 µL

[NOTE—The order of elution of peaks in the *Standard solution* is as follows. Internal standard peak, methylprednisolone hemisuccinate peak, and successive smaller peaks of free methylprednisolone and methylprednisolone 17-hemisuccinate.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methylprednisolone ($C_{22}H_{30}O_5$) in the portion of constituted Methylprednisolone Sodium Succinate for Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = ratio of the sum of the peak areas for methylprednisolone hemisuccinate and methylprednisolone 17-hemisuccinate to the peak area of the internal standard from the *Sample solution*

R_S = ratio of the sum of the peak areas for methylprednisolone hemisuccinate and methylprednisolone 17-hemisuccinate to the peak area of the internal standard from the *Standard solution*

C_S = concentration of USP Methylprednisolone Hemisuccinate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methylprednisolone in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of methylprednisolone, 374.47

M_{r2} = molecular weight of methylprednisolone hemisuccinate, 474.54

To this calculated amount, add the percentage of free methylprednisolone found in the test for *Free Methylprednisolone*.

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS• **FREE METHYLPREDNISOLONE****Analysis**

Samples: *Standard solution* and *Sample solution*

Using the chromatograms obtained in the *Assay*, measure the areas of the peaks from the internal standard and free methylprednisolone.

Calculate the percentage of free methylprednisolone in the portion of *Sample solution* taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of the free methylprednisolone to the internal standard from the *Sample solution*

R_S = peak area ratio of the free methylprednisolone to the internal standard from the *Standard solution*

- C_S = concentration of USP Methylprednisolone RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of methylprednisolone in the *Sample solution* (mg/mL)

Acceptance criteria: The amount of free methylprednisolone is NMT 6.6% of the labeled amount of methylprednisolone ($C_{22}H_{30}O_5$).

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

SPECIFIC TESTS

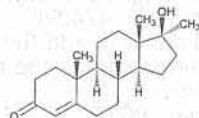
- **pH** (791)
Sample solution: 50 mg/mL of methylprednisolone sodium succinate
Acceptance criteria: 7.0–8.0
- **STERILITY TESTS** (71): Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.17 USP Endotoxin Units/mg of methylprednisolone
- **LOSS ON DRYING** (731)
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 2.0%
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions.*
- **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).
- **USP REFERENCE STANDARDS** (11)
 USP Endotoxin RS
 USP Fluorometholone RS
 USP Methylprednisolone RS
 USP Methylprednisolone Hemisuccinate RS

Methyltestosterone



$C_{20}H_{30}O_2$ 302.45
 Androst-4-en-3-one, 17-hydroxy-17-methyl-, (17 β)-;
 17 β -Hydroxy-17-methylandrost-4-en-3-one [58-18-4].

DEFINITION

Methyltestosterone contains NLT 97.0% and NMT 103.0% of methyltestosterone ($C_{20}H_{30}O_2$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile and water (55:45)

Standard stock solution: 0.25 mg/mL of USP

Methyltestosterone RS in methanol

Standard solution: 20 μ g/mL of USP Methyltestosterone RS in *Mobile phase* from the *Standard stock solution*

System suitability stock solution: 250 μ g/mL of USP

Testosterone RS in methanol

System suitability solution: Dilute 4 mL of the *System suitability stock solution* with the *Standard solution* to 50 mL.

Sample stock solution: 0.50 mg/mL of Methyltestosterone in methanol

Sample solution: 20 μ g/mL of Methyltestosterone in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 241 nm

Column: 4-mm \times 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 50 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for testosterone and methyltestosterone are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between testosterone and methyltestosterone, *System suitability solution*

Column efficiency: NLT 2000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.7, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methyltestosterone ($C_{20}H_{30}O_2$) in the portion of Methyltestosterone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of methyltestosterone from the *Sample solution*

r_S = peak response of methyltestosterone from the *Standard solution*

C_S = concentration of USP Methyltestosterone RS in the *Standard solution* (mg/mL)

C_U = concentration of Methyltestosterone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• ORGANIC IMPURITIES

Solution A: Methanol and water (55:45)

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	60	40
40	0	100
45	0	100
60	100	0

Sample solution: 0.5 mg/mL of Methyltestosterone in methanol

System suitability solution: 0.005 mg/mL of Methyltestosterone in methanol from the *Sample solution*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Flow rate:** 1 mL/min**Injection volume:** 5 μL**System suitability****Samples:** *Sample solution* and *System suitability solution***Suitability requirements****Relative standard deviation:** NMT 2.0%, *Sample solution***Signal-to-noise ratio:** NLT 100, *System suitability solution***Analysis****Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Methyltestosterone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_T = sum of all the peak responses from the *Sample solution*

[NOTE—Disregard any impurity peak less than 0.05%.]

Acceptance criteria**Any individual impurity:** NMT 0.5%**Total impurities:** NMT 1.0%**SPECIFIC TESTS**• **OPTICAL ROTATION**, *Specific Rotation* (7815)**Sample solution:** 10 mg/mL of Methyltestosterone in alcohol**Acceptance criteria:** +79° to +85°• **LOSS ON DRYING** (731)**Analysis:** Dry at 105° for 4 h.**Acceptance criteria:** NMT 2.0%**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.• **USP REFERENCE STANDARDS** (11)

USP Methyltestosterone RS

USP Testosterone RS

Methyltestosterone Capsules**DEFINITION**Methyltestosterone Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$).**IDENTIFICATION**• **A. INFRARED ABSORPTION****Sample:** Evaporate to dryness 25 mL of the *Sample stock solution* from the Assay.**Acceptance criteria:** The IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima at the same wavelengths as those of a similar preparation of USP Methyltestosterone RS.**ASSAY**• **PROCEDURE****Standard solution:** 10 μg/mL of USP Methyltestosterone RS in alcohol**Sample stock solution:** Nominally 0.2 mg/mL of methyltestosterone prepared as follows. Transfer the equivalent to 10 mg of methyltestosterone from the contents of NLT 20 Capsules to a 125-mL separator with the aid of about 5 mL of water. Extract with four

20-mL portions of chloroform, filtering each through chloroform-washed cotton. Evaporate the combined extracts on a steam bath, with the aid of a current of air, to dryness. Dissolve the residue in alcohol, transfer to a 50-mL volumetric flask, and dilute with alcohol to volume.

Sample solution: Nominally 10 μg/mL of methyltestosterone in alcohol from the *Sample stock solution***Instrumental conditions****Mode:** UV**Analytical wavelength:** Maximum absorbance at about 241 nm**Cell:** 1 cm**Blank:** Alcohol**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$) in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of USP Methyltestosterone RS in the *Standard solution* (μg/mL) C_U = nominal concentration of methyltestosterone in the *Sample solution* (μg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**• **DISSOLUTION** (711)**Medium:** Water; 900 mL**Apparatus 1:** 100 rpm**Time:** 45 min**Standard solution:** 10 μg/mL of USP Methyltestosterone RS in *Medium***Sample solution:** Filter a portion of the solution under test. Dilute with *Medium* to obtain a solution containing about 10 μg/mL of methyltestosterone.**Instrumental conditions****Mode:** UV**Analytical wavelength:** Maximum absorbance at about 248 nm**Blank:** *Medium***Analysis****Samples:** *Standard solution* and *Sample solution***Tolerances:** NLT 70% (Q) of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$) is dissolved.• **UNIFORMITY OF DOSAGE UNITS** (905)**Procedure for content uniformity****Standard solution:** 0.010 mg/mL of USP Methyltestosterone RS in methanol**Sample stock solution:** Transfer the contents of 1 Capsule to a 100-mL volumetric flask. Add 50 mL of methanol, and shake by mechanical means for 60 min. Dilute with methanol to volume, and filter, discarding the first 20 mL of the filtrate.**Sample solution:** Dilute a suitable volume of the *Sample stock solution* with methanol to obtain 0.010 mg/mL of methyltestosterone.**Instrumental conditions****Mode:** UV**Analytical wavelength:** Maximum absorbance at about 241 nm**Cell:** 1 cm**Blank:** Methanol**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

- A_U = absorbance of methyltestosterone from the *Sample solution*
 A_S = absorbance of methyltestosterone from the *Standard solution*
 C_S = concentration of USP Methyltestosterone RS in the *Standard solution* (mg/mL)
 L = label claim (mg/Capsule)
 V = volume of the *Sample solution* (mL)
 D = dilution factor of the *Sample solution*
 Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
USP Methyltestosterone RS

Methyltestosterone Tablets

DEFINITION

Methyltestosterone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$).

IDENTIFICATION• **A. INFRARED ABSORPTION**

Sample: Evaporate to dryness 25 mL of the *Sample stock solution* from the *Assay*.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima at the same wavelengths as those of a similar preparation of USP Methyltestosterone RS.

ASSAY• **PROCEDURE**

Standard solution: 10 µg/mL of USP Methyltestosterone RS in alcohol

Sample stock solution: Nominally 0.2 mg/mL of methyltestosterone prepared as follows. Transfer the equivalent to 10 mg of methyltestosterone from NLT 20 powdered Tablets to a 125-mL separator with the aid of about 5 mL of water. Extract with four 25-mL portions of chloroform, filtering each through chloroform-washed cotton. Evaporate the combined extracts on a steam bath, with the aid of a current of air, to dryness. Dissolve the residue in alcohol, transfer to a 50-mL volumetric flask, and dilute with alcohol to volume.

Sample solution: Nominally 10 µg/mL of methyltestosterone in alcohol from the *Sample stock solution*

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 241 nm

Cell: 1 cm

Blank: Alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

- A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of USP Methyltestosterone RS in the *Standard solution* (µg/mL)
 C_U = nominal concentration of methyltestosterone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISINTEGRATION (701)**

Time: 30 min

Acceptance criteria: Tablets intended for buccal administration meet the requirements for *Buccal Tablets*.

• **UNIFORMITY OF DOSAGE UNITS (905)****Procedure for content uniformity**

Standard solution: 0.010 mg/mL of USP Methyltestosterone RS in methanol

Sample stock solution: Transfer 1 finely powdered Tablet to a 100-mL volumetric flask. Add 50 mL of methanol, and shake by mechanical means for 60 min. Dilute with methanol to volume, and filter, discarding the first 20 mL of the filtrate.

Sample solution: Dilute a suitable volume of the *Sample stock solution* with methanol to obtain 0.010 mg/mL of methyltestosterone.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 241 nm

Cell: 1 cm

Blank: Methanol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$) in the Tablet taken:

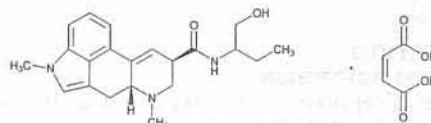
$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

- A_U = absorbance of methyltestosterone from the *Sample solution*
 A_S = absorbance of methyltestosterone from the *Standard solution*
 C_S = concentration of USP Methyltestosterone RS in the *Standard solution* (mg/mL)
 L = label claim (mg/Tablet)
 V = volume of the *Sample solution* (mL)
 D = dilution factor of the *Sample solution*
 Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
USP Methyltestosterone RS

Methysergide Maleate



$C_{21}H_{27}N_3O_2 \cdot C_4H_4O_4$ 469.53
 Ergoline-8-carboxamide, 9,10-didehydro-N-[1-(hydroxymethyl)propyl]-1,6-dimethyl-, (8β)-, (Z)-2-butenedioate (1:1) (salt);
 9,10-Didehydro-N-[1-(hydroxymethyl)propyl]-1,6-dimethylethylergoline-8β-carboxamide maleate (1:1) (salt) [129-49-7].

DEFINITION

Methysergide Maleate contains NLT 97.0% and NMT 103.0% of methysergide maleate ($C_{21}H_{27}N_3O_2 \cdot C_4H_4O_4$), calculated on the dried basis.

IDENTIFICATION• **A. INFRARED ABSORPTION** (197K)• **B. THIN-LAYER CHROMATOGRAPHY**

Conduct this test without exposure to daylight and with minimum exposure to artificial light.

Standard solution: 5 mg/mL of USP Methysergide Maleate RS in methanol

Sample solution: 5 mg/mL of Methysergide Maleate in methanol

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 25 μ L

Developing solvent: Chloroform and methanol (20:1)

Spray reagent: 8 mg/mL of *p*-dimethylaminobenzaldehyde in a cooled mixture of alcohol and sulfuric acid (8:2)

Analysis

Samples: *Standard solution* and *Sample solution*

In the chromatographic chamber, place a volume of the *Developing solvent* sufficient to develop the chromatogram. Place a beaker containing 25 mL of ammonium hydroxide in the chamber, cover, and allow to equilibrate for 30 min. Apply the *Samples*, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent*. Allow the plate to dry, then expose it briefly to fumes of a mixture of nitric and hydrochloric acids.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY• **PROCEDURE**

Sample solution: 200 mg of Methysergide Maleate in 30 mL of glacial acetic acid. Add 1 drop of crystal violet TS.

Analysis: Titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 46.95 mg of methysergide maleate ($C_{21}H_{27}N_3O_2 \cdot C_4H_4O_4$).

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES• **ORDINARY IMPURITIES** (466)

Standard solution and Sample solution: Methanol

Eluant: Use the *Developing solvent* from *Identification* test B.

Visualization: 1

Acceptance criteria: Meets the requirements

SPECIFIC TESTS• **OPTICAL ROTATION, Specific Rotation** (781S)

Sample solution: 2.5 mg/mL in water

Acceptance criteria: +35° to +45°

• **PH** (791)

Sample solution: 1 in 500 in carbon dioxide-free water

Acceptance criteria: 3.7–4.7

• **LOSS ON DRYING** (731)

Analysis: Dry a sample under vacuum at 120° for 2 h.

Acceptance criteria: NMT 7.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, in a cold place.

• **USP REFERENCE STANDARDS** (11)

USP Methysergide Maleate RS

Methysergide Maleate Tablets**DEFINITION**

Methysergide Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methysergide maleate ($C_{21}H_{27}N_3O_2 \cdot C_4H_4O_4$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Conduct this procedure with a minimum exposure to light.

Mobile phase: Dissolve 6.8 g of monobasic potassium phosphate in 700 mL of water, add 300 mL of acetonitrile, and mix.

Diluent: Methanol and 10 g/L of tartaric acid (50:50)

Standard solution: 0.1 mg/mL of USP Methysergide Maleate RS in *Diluent*

[NOTE—Sonication may be used.]

Sample solution: Nominally 0.1 mg/mL of methysergide maleate prepared as follows. Transfer a portion of NLT 20 finely powdered Tablets equivalent to NLT 10 mg of methysergide maleate to a suitable volumetric flask. Add 75% of the flask volume with *Diluent*. Shake by mechanical means for 60 min. Dilute with *Diluent* to volume. Filter, and discard the first 20 mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 318 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Flow rate: 2 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between the analyte and the closest adjacent peak

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methysergide maleate

($C_{21}H_{27}N_3O_2 \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Methysergide Maleate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of methysergide maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)

Medium: Tartaric acid solution (1 in 200); 900 mL

Apparatus 2: 100 rpm

Time: 30 min

Standard solution: 0.002 mg/mL of USP Methysergide Maleate RS in *Medium*

Sample solution: Portions of the solution under test suitably diluted with *Medium*

Instrumental conditions

Mode: Fluorescence
 Excitation wavelength: 327 nm
 Emission wavelength: 428 nm
 Blank: Tartaric acid solution (1 in 200)

Analysis

Samples: *Standard solution* and *Sample solution*
 Calculate the percentage of the labeled amount of methysergide maleate ($C_{21}H_{27}N_3O_2 \cdot C_4H_4O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

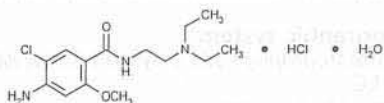
A_U = absorbance from the *Sample solution*
 A_S = absorbance from the *Standard solution*
 C_S = concentration of USP Methysergide Maleate RS in the *Standard solution* (mg/mL)
 V = volume of *Medium*, 900 mL
 L = label claim of methysergide maleate (mg/Tablet)

Tolerances: NLT 70% (Q) of the labeled amount of methysergide maleate ($C_{21}H_{27}N_3O_2 \cdot C_4H_4O_4$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store below 30°.
- **USP REFERENCE STANDARDS** (11)
 USP Methysergide Maleate RS

Metoclopramide Hydrochloride

$C_{14}H_{22}ClN_3O_2 \cdot HCl \cdot H_2O$ 354.27
 Benzamide, 4-amino-5-chloro-N-[2-(diethylamino)ethyl]-2-methoxy-, monohydrochloride, monohydrate;
 4-Amino-5-chloro-N-[2-(diethylamino)ethyl]-o-anisamide monohydrochloride monohydrate [54143-57-6].

DEFINITION

Metoclopramide Hydrochloride contains NLT 98.0% and NMT 101.0% of metoclopramide hydrochloride ($C_{14}H_{22}ClN_3O_2 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197)
 [NOTE—Methods described in *Infrared Absorption* (197K), (197M), or (197A) may be used.]
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191)
Sample solution: Dissolve 100 mg in 2 mL of water, and acidify the solution with dilute nitric acid.
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
Sample: 250 mg
Analysis: Dissolve the *Sample* in a mixture of 5.0 mL of 0.01 N hydrochloric acid and 50 mL of alcohol. Titrate with 0.1 N sodium hydroxide VS (see *Titrimetry* (541)), determining the endpoint potentiometrically. Read the volume of 0.1 N sodium hydroxide added between the two points of inflection. Each mL of 0.1 N sodium hydroxide is equivalent to 33.63 mg of the anhydrous metoclopramide hydrochloride ($C_{14}H_{22}ClN_3O_2 \cdot HCl$).
Acceptance criteria: 98.0%–101.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **ORGANIC IMPURITIES**
Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 700 mL of water. Add 0.2 mL of *N,N*-dimethyloctylamine, and adjust with dilute phosphoric acid to a pH of 4.0. Dilute with water to 1000 mL.
Mobile phase: Acetonitrile and *Buffer* (250:1000)
Standard stock solution: 0.1 mg/mL each of USP Metoclopramide Hydrochloride RS, USP Metoclopramide Related Compound A RS, USP Metoclopramide Related Compound B RS, and USP Metoclopramide Related Compound D RS in *Mobile phase*
Standard solution: 2.0 µg/mL each of USP Metoclopramide Hydrochloride RS, USP Metoclopramide Related Compound A RS, USP Metoclopramide Related Compound B RS, and USP Metoclopramide Related Compound D RS in *Mobile phase* from the *Standard stock solution*

Sample solution: 1.0 mg/mL of Metoclopramide Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: At least 8 times the retention time of the metoclopramide peak

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 for metoclopramide related compound A and metoclopramide

Analysis

Samples: *Standard solution* and *Sample solution*
 Calculate the percentage of each of metoclopramide related compounds A, B, and D in the portion of Metoclopramide Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the relevant metoclopramide related compound from the *Sample solution*
 r_S = peak response of the relevant metoclopramide related compound from the *Standard solution*
 C_S = concentration of the relevant USP Metoclopramide Related Compound RS in the *Standard solution* (mg/mL)
 C_U = concentration of Metoclopramide Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Metoclopramide Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*
 r_S = peak response of metoclopramide from the *Standard solution*
 C_S = concentration of USP Metoclopramide Hydrochloride RS in the *Standard solution* (mg/mL)
 C_U = concentration of Metoclopramide Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
N-Acetylmeclopramide (meclopramide related compound A) ^a	0.8	0.15
Metoclopramide	1.0	—
Methyl 4-acetamido-2-methoxybenzoate (meclopramide related compound D)	2.4–2.7	0.15
N-Acetyl methyl ester analog (meclopramide related compound B) ^b	5.2–6.7	0.15
Any other individual impurity	—	0.10
Total impurities	—	0.50

^a 4-Acetamido-5-chloro-N-[2-(diethylamino)ethyl]-2-methoxybenzamide.

^b Methyl 4-acetamido-5-chloro-2-methoxybenzoate.

SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* (921): 4.5%–6.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers. Store at room temperature.
- **USP REFERENCE STANDARDS** (11)
 - USP Metoclopramide Hydrochloride RS
 - USP Metoclopramide Related Compound A RS
 - N-Acetylmeclopramide.
 - 4-Acetamido-5-chloro-N-[2-(diethylamino)ethyl]-2-methoxybenzamide.
 - C₁₆H₂₄ClN₃O₃ 341.83
 - USP Metoclopramide Related Compound B RS
 - N-Acetyl methyl ester analog.
 - Methyl 4-acetamido-5-chloro-2-methoxybenzoate.
 - C₁₁H₁₂ClNO₄ 257.67
 - USP Metoclopramide Related Compound D RS
 - Methyl 4-acetamido-2-methoxybenzoate.
 - C₁₁H₁₃NO₄ 223.23

Metoclopramide Injection

DEFINITION

Metoclopramide Injection is a sterile solution of Metoclopramide Hydrochloride in Water for Injection. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of metoclopramide (C₁₄H₂₂ClN₃O₂).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Mobile phase: Dissolve 2.7 g of sodium acetate in 500 mL of water. Add 500 mL of acetonitrile and 2 mL of tetramethylammonium hydroxide solution in methanol (1 in 5), and mix. Adjust with glacial acetic acid to a pH of 6.5, filter, and degas.

System suitability stock solution: Transfer 12.5 mg of benzenesulfonamide to a 25-mL volumetric flask. Add 15 mL of methanol, and shake to dissolve. Dilute with 0.01 M phosphoric acid to volume.

Standard stock solution: 0.9 mg/mL of USP Metoclopramide Hydrochloride RS in 0.01 M phosphoric acid

System suitability solution: Transfer 5 mL of *System suitability stock solution* and 5 mL of *Standard stock solution* into a 100-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume.

Standard solution: 45 µg/mL of USP Metoclopramide Hydrochloride RS (equivalent to 40 µg/mL of metoclopramide) from *Standard stock solution*. Dilute with 0.01 M phosphoric acid.

Sample solution: Nominally 40 µg/mL of metoclopramide, prepared as follows. Transfer a volume of *Injection*, equivalent to about 40 mg of metoclopramide, to a 100-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm or diode array. [NOTE—Use the diode array detector to perform *Identification test B*.]

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

[NOTE—The relative retention times for benzenesulfonamide and metoclopramide are 0.7 and 1.0, respectively.]

Resolution: NLT 1.5 between the benzenesulfonamide and metoclopramide peaks, *System suitability solution*

Tailing factor: NMT 2.0 for the metoclopramide peak, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metoclopramide (C₁₄H₂₂ClN₃O₂) in the portion of *Injection* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Metoclopramide Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metoclopramide in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of metoclopramide, 299.80

M_{r2} = molecular weight of anhydrous metoclopramide hydrochloride, 336.26

Acceptance criteria: 90.0%–110.0%

IMPURITIES

ORGANIC IMPURITIES

Mobile phase: Prepare a 1.88 g/L solution of sodium 1-hexanesulfonate solution (0.01 M solution) in a mixture of acetonitrile and water (60:40), and adjust with glacial acetic acid to a pH of 4.0.

Standard solution: 5.5 µg/mL of USP Metoclopramide Hydrochloride RS in *Mobile phase*

Sample solution: Dilute a volume of *Injection* with *Mobile phase* to obtain a solution containing about 1.0 mg/mL of metoclopramide.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 265 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Flow rate:** 2 mL/min**Injection volume:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.8**Relative standard deviation:** NMT 5.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_S = peak response of metoclopramide from the *Standard solution* C_S = concentration of USP Metoclopramide Hydrochloride RS in the *Standard solution* (μg/mL) C_U = nominal concentration of metoclopramide in the *Sample solution* (μg/mL) M_{r1} = molecular weight of metoclopramide, 299.80 M_{r2} = molecular weight of anhydrous metoclopramide hydrochloride, 336.26**Acceptance criteria:** NMT 0.5% of any individual impurity is found.**SPECIFIC TESTS**

- pH** (791): 2.5–6.5
- BACTERIAL ENDOTOXINS TEST** (85): NMT 2.5 USP Endotoxin Units/mg of metoclopramide
- PARTICULATE MATTER IN INJECTIONS** (788): It meets the requirements for small-volume injections.
- OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. [NOTE—Injection containing an antioxidant agent does not require protection from light.] Store at controlled room temperature.
- USP REFERENCE STANDARDS** (11)
 - USP Endotoxin RS
 - USP Metoclopramide Hydrochloride RS

Metoclopramide Oral Solution**DEFINITION**Metoclopramide Oral Solution contains an amount of metoclopramide hydrochloride ($C_{14}H_{22}ClN_3O_2 \cdot HCl \cdot H_2O$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$).**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**PROCEDURE****Mobile phase:** Dissolve 2.7 g of sodium acetate in 600 mL of water, and add 400 mL of acetonitrile and 4 mL of tetramethylammonium hydroxide solution in methanol (25%). Adjust with glacial acetic acid to a pH of 6.5, filter, and degas.**System suitability stock solution:** Transfer 125 mg of benzenesulfonamide to a 25-mL volumetric flask. Add 15 mL of methanol, and shake to dissolve. Dilute with 0.01 M phosphoric acid to volume.**Standard stock solution:** 9 mg/mL of USP

Metoclopramide Hydrochloride RS in 0.01 M phosphoric acid

System suitability solution: Transfer 15 mL of *System suitability stock solution* and 5 mL of *Standard stock solution* into a 250-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume.**Standard solution:** 180 μg/mL of USP Metoclopramide Hydrochloride RS (equivalent to 160 μg/mL of metoclopramide) from *Standard stock solution*. Dilute with 0.01 M phosphoric acid.**Sample solution:** Nominally 160 μg/mL of metoclopramide, prepared as follows. Transfer a volume of Oral Solution, equivalent to about 4 mg of metoclopramide, to a 25-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 215 nm or diode array. [NOTE—Use the diode array detector to perform *Identification test B*.]**Column:** 4.6-mm × 25-cm; packing L1**Flow rate:** 1.5 mL/min**Injection volume:** 20 μL**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements**

[NOTE—The relative retention times for benzenesulfonamide and metoclopramide are 0.2 and 1.0, respectively.]

Resolution: NLT 1.5 between the benzenesulfonamide and metoclopramide peaks, *System suitability solution***Tailing factor:** NMT 2.0 for the metoclopramide peak, *Standard solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration from USP Metoclopramide Hydrochloride RS in the *Standard solution* (μg/mL) C_U = nominal concentration of metoclopramide in the *Sample solution* (μg/mL) M_{r1} = molecular weight of metoclopramide, 299.80 M_{r2} = molecular weight of anhydrous metoclopramide hydrochloride, 336.26**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS****UNIFORMITY OF DOSAGE UNITS** (905)For oral solution packaged in single-unit containers:
Meets the requirements

- **DELIVERABLE VOLUME** (698)

For oral solution packaged in multiple-unit containers: Meets the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase: Prepare a 1.88 g/L solution of sodium 1-hexanesulfonate solution (0.01 M solution) in a mixture of acetonitrile and water (60:40), and adjust with glacial acetic acid to a pH of 4.0.

Standard solution: 5.5 µg/mL of USP Metoclopramide Hydrochloride RS in *Mobile phase*

Sample solution: Dilute a volume of Oral Solution with *Mobile phase* to obtain a solution containing about 1.0 mg/mL of metoclopramide.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.8

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of metoclopramide from the *Standard solution*

C_S = concentration of USP Metoclopramide Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metoclopramide in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of metoclopramide, 299.80

M_{r2} = molecular weight of anhydrous metoclopramide hydrochloride, 336.26

Acceptance criteria: NMT 0.5% of any individual impurity is found. Disregard any peak with a relative retention time of 0.5 or less.

SPECIFIC TESTS

- **pH** (791): 2.0–5.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature. Protect from freezing.

- **USP REFERENCE STANDARDS** (11)

USP Metoclopramide Hydrochloride RS

Metoclopramide Tablets

DEFINITION

Metoclopramide Tablets contain an amount of metoclopramide hydrochloride ($C_{14}H_{22}ClN_3O_2 \cdot HCl \cdot H_2O$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 2.7 g of sodium acetate in 500 mL of water. Add 500 mL of acetonitrile and 2 mL of tetramethylammonium hydroxide solution in methanol (1 in 5), and mix. Adjust with glacial acetic acid to a pH of 6.5, filter, and degas.

System suitability stock solution: Transfer 12.5 mg of benzenesulfonamide to a 25-mL volumetric flask. Add 15 mL of methanol, and shake to dissolve. Dilute with 0.01 M phosphoric acid to volume.

Standard stock solution: 0.9 mg/mL of USP Metoclopramide Hydrochloride RS in 0.01 M phosphoric acid

System suitability solution: Transfer 5 mL of *System suitability stock solution* and 5 mL of *Standard stock solution* into a 100-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume.

Standard solution: 45 µg/mL of USP Metoclopramide Hydrochloride RS (equivalent to 40 µg/mL of metoclopramide) from *Standard stock solution* diluted with 0.01 M phosphoric acid

Sample solution: Nominally 40 µg/mL of metoclopramide, prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of metoclopramide, to a 100-mL volumetric flask, add about 70 mL of 0.01 M phosphoric acid, and sonicate for 5 min. Cool to room temperature, dilute with 0.01 M phosphoric acid to volume, and mix. Pass the solution through a filter of 0.45-µm pore size, discarding the first portion of the filtrate. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with 0.01 M phosphoric acid to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm or diode array. [NOTE—Use the diode array detector to perform *Identification test B*.]

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

[NOTE—The relative retention times for benzenesulfonamide and metoclopramide are 0.7 and 1.0, respectively.]

Resolution: NLT 1.5 between the benzenesulfonamide and metoclopramide peaks, *System suitability solution*

Tailing factor: NMT 2.0 for the metoclopramide peak, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Metoclopramide Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metoclopramide in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of metoclopramide, 299.80
 M_{r2} = molecular weight of anhydrous metoclopramide hydrochloride, 336.26
 Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)

Medium: Water; 900 mL

Apparatus 1: 50 rpm

Time: 30 min

Standard solution: USP Metoclopramide Hydrochloride RS at a known concentration in *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium*

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: Wavelength of maximum absorbance at about 309 nm

Tolerances: NLT 75% (Q) of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements**IMPURITIES**• **ORGANIC IMPURITIES**

Mobile phase: Prepare a 1.88 g/L solution of sodium 1-hexanesulfonate solution (0.01 M solution) in a mixture of acetonitrile and water (60:40), and adjust with glacial acetic acid to a pH of 4.0.

Standard solution: 5.5 µg/mL of USP Metoclopramide Hydrochloride RS in *Mobile phase*

Sample solution: Shake a quantity of the powdered Tablets containing the equivalent of 100 mg of metoclopramide with 20 mL of methanol for 5 min, and pass through a suitable filter, discarding the first few mL of the filtrate. Dilute a portion of the filtrate with *Mobile phase* to obtain a solution containing about 1.0 mg/mL of metoclopramide.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.8

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of metoclopramide from the *Standard solution*

C_S = concentration of USP Metoclopramide Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metoclopramide in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of metoclopramide, 299.80

M_{r2} = molecular weight of anhydrous metoclopramide hydrochloride, 336.26

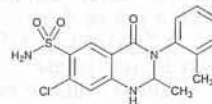
Acceptance criteria: NMT 0.5% of any individual impurity is found.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Metoclopramide Hydrochloride RS

Metolazone

$C_{16}H_{16}ClN_3O_3S$

365.83

6-Quinazolinesulfonamide, 7-chloro-1,2,3,4-tetrahydro-2-methyl-3-(2-methylphenyl)-4-oxo-;

7-Chloro-1,2,3,4-tetrahydro-2-methyl-4-oxo-3-o-tolyl-6-quinazolinesulfonamide [17560-51-9].

DEFINITION

Metolazone contains NLT 97.0% and NMT 102.0% of metolazone ($C_{16}H_{16}ClN_3O_3S$), calculated on the dried basis.

IDENTIFICATION• **A. INFRARED ABSORPTION** (197K)• **B. ULTRAVIOLET ABSORPTION** (197U)

Sample solution: 5 µg/mL in methanol

Acceptance criteria: Meets the requirements

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**ASSAY**• **PROCEDURE**

Protect all solutions of Metolazone from light.

Buffer: 5.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile, methanol, and *Buffer* (10:25:65)

Standard stock solution: 0.5 mg/mL of USP Metolazone RS in tetrahydrofuran

Standard solution: 0.05 mg/mL of USP Metolazone RS in alcohol from the *Standard stock solution*

Sample stock solution: 1 mg/mL of Metolazone in tetrahydrofuran

Sample solution: 0.05 mg/mL of Metolazone in alcohol from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.4

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of metolazone

($C_{16}H_{16}ClN_3O_3S$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metolazone from the *Sample solution*

r_S = peak response of metolazone from the *Standard solution*

C_S = concentration of USP Metolazone RS in the *Standard solution* (µg/mL)

C_U = concentration of Metolazone in the *Sample solution* ($\mu\text{g/mL}$)
 Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS**, *Method II* (231): 15 ppm (Official 1-Jan-2018)

• **ORGANIC IMPURITIES**

Buffer and Mobile phase: Proceed as directed in the *Assay*.

Standard stock solution: 0.48 mg/mL of USP Metolazone RS in tetrahydrofuran

Standard solution: 6 $\mu\text{g/mL}$ of USP Metolazone RS in alcohol from the *Standard stock solution*

Sample solution: 0.6 mg/mL of Metolazone, prepared as follows. Dissolve a suitable amount of Metolazone with tetrahydrofuran in 50% of the total volume, and dilute with alcohol to volume.

Chromatographic system: Proceed as directed in the *Assay*, except for the following:

Column: 4.6-mm \times 25-cm; 5- μm packing 1

Run time: NLT 3.5 times the retention time of metolazone

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.4

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of metolazone from the *Standard solution*

C_S = concentration of USP Metolazone RS in the *Standard solution* (mg/mL)

C_U = concentration of Metolazone in the *Sample solution* (mg/mL)

F = relative response factor of each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desmethyl metolazone ^a	0.7	1.0	0.5
Metolazone benzamide analog ^b	0.8	0.83	0.5
Metolazone	1.0	1.0	—
meta-Metolazone ^c	1.3	0.91	0.5
para-Metolazone ^d	1.4	0.91	0.5
Didehydrometolazone ^e	1.5	0.83	0.5

^a 7-Chloro-2-methyl-4-oxo-3-phenyl-1,2,3,4-tetrahydroquinazoline-6-sulfonamide.

^b 2-Amino-4-chloro-5-sulfamoyl-N-(o-tolyl)benzamide.

^c 7-Chloro-2-methyl-4-oxo-3-(m-tolyl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide.

^d 7-Chloro-2-methyl-4-oxo-3-(p-tolyl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide.

^e 7-Chloro-2-methyl-4-oxo-3-(o-tolyl)-3,4-dihydroquinazoline-6-sulfonamide.

^f Sum of all individual impurities. Disregard any peaks less than 0.05%.

Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other individual impurity	—	—	0.10
Total impurities ^f	—	—	1.0

^a 7-Chloro-2-methyl-4-oxo-3-phenyl-1,2,3,4-tetrahydroquinazoline-6-sulfonamide.

^b 2-Amino-4-chloro-5-sulfamoyl-N-(o-tolyl)benzamide.

^c 7-Chloro-2-methyl-4-oxo-3-(m-tolyl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide.

^d 7-Chloro-2-methyl-4-oxo-3-(p-tolyl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide.

^e 7-Chloro-2-methyl-4-oxo-3-(o-tolyl)-3,4-dihydroquinazoline-6-sulfonamide.

^f Sum of all individual impurities. Disregard any peaks less than 0.05%.

SPECIFIC TESTS• **LOSS ON DRYING** (731)

Analysis: Dry a sample at 105° for 2 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** (11)

USP Metolazone RS

Metolazone Compounded Oral Suspension

DEFINITION

Metolazone Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of metolazone ($\text{C}_{16}\text{H}_{16}\text{ClN}_3\text{O}_3\text{S}$).

Prepare Metolazone Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Metolazone	100 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

Place the required number of tablets in a suitable mortar and comminute to a fine powder, or use *Metolazone* powder. Add 20 mL of *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions, and transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add *Vehicle* in portions to rinse the mortar, then add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY• **PROCEDURE**

Mobile phase: Methanol and water (70:30) containing 1.5 g/L of ammonium acetate and 1 mL/L of diisopropylamine. Filter, and degas.

Standard solution: 1.0 $\mu\text{g/mL}$ of USP Metolazone RS

Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 1.0 mL of the sample to a 1000-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 20-cm; 5-μm packing L3**Flow rate:** 1.0 mL/min**Injection volume:** 20 μL**System suitability****Sample:** *Standard solution*

[NOTE—The retention time for metolazone is about 6.0 min.]

Suitability requirements**Relative standard deviation:** NMT 2.2% for replicate injections**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of metolazone (C₁₆H₁₆ClN₃O₃S) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Metolazone RS in the *Standard solution* (μg/mL) C_U = nominal concentration of metolazone in the *Sample solution* (μg/mL)**Acceptance criteria:** 90.0%–110.0%**SPECIFIC TESTS**

- **PH** <791>: 3.6–4.6

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature, or in a refrigerator
- **LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** (11)
USP Metolazone RS

Metolazone Tablets**DEFINITION**Metolazone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metolazone (C₁₆H₁₆ClN₃O₃S).**IDENTIFICATION**

- **A. ULTRAVIOLET ABSORPTION** (197U)

Sample solution: Dilute 3 mL of the *Sample solution* in the *Assay* with methanol to 25 mL.**Acceptance criteria:** Meet the requirements**ASSAY**

- **PROCEDURE**

[NOTE—Use low-actinic glassware throughout the *Assay*.]**Buffer:** 1.38 g of monobasic potassium phosphate monohydrate in 900 mL of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 1000 mL.**Mobile phase:** Methanol, acetonitrile, and *Buffer* (28:7:65)**Standard stock solution:** 0.25 mg/mL of USP Metolazone RS in methanol**Standard solution:** 5 μg/mL of USP Metolazone RS in *Mobile phase* from *Standard stock solution***Sample stock solution:** Transfer 10 Tablets to a 200-mL volumetric flask. Add 3 mL of water and 100 mL of methanol, and sonicate for 30 min. If disintegration is not complete, sonicate for an additional 30 min. Shake by mechanical means for 30 min. Dilute with methanol to volume.**Sample solution:** Nominally equivalent to 5 μg/mL of metolazone in *Mobile phase* from the *Sample stock solution***Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 235 nm**Column:** 3.9-mm × 15-cm; packing L1**Flow rate:** 1.1 mL/min**Injection volume:** 100 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of metolazone (C₁₆H₁₆ClN₃O₃S) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Metolazone RS in the *Standard solution* (μg/mL) C_U = nominal concentration of the *Sample solution* (μg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**

- **DISSOLUTION** <711>

[NOTE—Protect all solutions from light.]

Test 1**Medium:** 2% w/v sodium lauryl sulfate in 0.05 M monobasic sodium phosphate. Heat the mixture to about 37° to dissolve the sodium lauryl sulfate, and adjust with 10 N sodium hydroxide to a pH of 7.5; 900 mL, deaerated**Apparatus 2:** 75 rpm**Time:** 120 min**Buffer:** 0.05 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.00.**Mobile phase:** Acetonitrile, methanol, and *Buffer* (270:50:680)**Standard stock solution:** 0.28 mg/mL of USP Metolazone RS. Initially add methanol to 2% of the volume of the flask. Sonicate to dissolve, and dilute with *Medium* to volume.**Standard solution:** (L/900) mg/mL in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection volume: 50 μL

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 2000 theoretical plates

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of metolazone (C₁₆H₁₆ClN₃O₃S) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of the *Standard solution* (mg/mL) L = label claim (mg/Tablet) V = volume of *Medium*, 900 mL**Tolerances:** NLT 75% (Q) of the labeled amount of metolazone (C₁₆H₁₆ClN₃O₃S) is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.**Medium:** Prepare a solution of 0.05 M dibasic sodium phosphate in a suitable flask, and adjust with phosphoric acid to a pH of 7.5. Dissolve a suitable amount of sodium lauryl sulfate to obtain a 20-g/L solution; 900 mL**Apparatus 2:** 75 rpm**Time:** 120 min**Standard stock solution:** 0.275 mg/mL of USP Metolazone RS. Initially add methanol to 10% of the volume of the flask. Sonicate to dissolve, and dilute with *Medium* to volume.**Standard solution:** (L/900) mg/mL in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

Detector: UV 238 nm

Path length: 1 cm

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of metolazone (C₁₆H₁₆ClN₃O₃S) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of the *Standard solution* (mg/mL) L = label claim (mg/Tablet) V = volume of *Medium*, 900 mL**Tolerances:** NLT 75% (Q) of the labeled amount of metolazone (C₁₆H₁₆ClN₃O₃S) is dissolved.

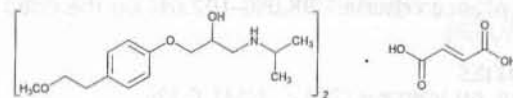
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store below 30°.
- **LABELING:** When more than one test for *Dissolution* is given, the *Labeling* section states the test for *Dissolution* used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS (11)**

USP Metolazone RS

Metoprolol Fumarate(C₁₅H₂₅NO₃)₂ · C₄H₄O₄

650.80

2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-

[(1-methylethyl)amino]-, (±)-, (E)-2-butanedioate (2:1) (salt);

(±)-1-(Isopropylamino)-3-[p-(2-methoxyethyl)phenoxy]-2-propanol fumarate (2:1) (salt) [119637-66-0].

DEFINITION**Change to read:**Metoprolol Fumarate contains ▲NLT 98.0% and NMT 102.0%▲^{USP40} of metoprolol fumarate [(C₁₅H₂₅NO₃)₂ · C₄H₄O₄], calculated on the dried basis.**IDENTIFICATION**• **A. INFRARED ABSORPTION (197K)****Change to read:**

- **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲^{USP40}

ASSAY**Change to read:**• **PROCEDURE**▲**Solution A:** 1.3 g/L of sodium dodecyl sulfate in 0.1% (w/v) phosphoric acid**Solution B:** Acetonitrile**Mobile phase:** *Solution A* and *Solution B* (60:40)**Standard solution:** 1 mg/mL of USP Metoprolol Fumarate RS in *Mobile phase***Sample solution:** 1 mg/mL of Metoprolol Fumarate in *Mobile phase***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 223 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 μL

Run time: 10 min

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 0.73%

AnalysisSamples: *Standard solution* and *Sample solution*

Calculate the percentage of metoprolol fumarate

[(C₁₅H₂₅NO₃)₂ · C₄H₄O₄] in the portion of Metoprolol Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of metoprolol from the *Sample solution*
 r_S = peak response of metoprolol from the *Standard solution*
 C_S = concentration of USP Metoprolol Fumarate RS in the *Standard solution* (mg/mL)
 C_U = concentration of Metoprolol Fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis▲USP40

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method I** (231): NMT 10 ppm● (Official 1-Jan-2018)

Change to read:

• **ORGANIC IMPURITIES**

▲Solution A: 1.3 g/L of sodium dodecyl sulfate in 0.1% (w/v) phosphoric acid

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
8	60	40
13	10	90
13.1	60	40
16	60	40

Diluent: Solution A and Solution B (60:40)

System suitability solution: 5 µg/mL each of USP

Metoprolol Fumarate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, and USP Metoprolol Related Compound C RS in Diluent

Standard solution: 2.5 µg/mL each of USP Metoprolol Fumarate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, USP Metoprolol Compound C RS, and USP Metoprolol Related Compound D RS in Diluent

Sample solution: 1 mg/mL of Metoprolol Fumarate in Diluent

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 223 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: System suitability solution and Standard solution

Suitability requirements

Resolution: NLT 1.5 between metoprolol related compound A and metoprolol related compound B; NLT 2.5 between metoprolol related compound B and metoprolol related compound C, System suitability solution

Relative standard deviation: NMT 2.0% for metoprolol, metoprolol related compound A, metoprolol related compound B, metoprolol related compound C, and metoprolol related compound D, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the corresponding metoprolol related compound in the portion of Metoprolol Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding metoprolol related compound in the *Sample solution*

r_S = peak response of the corresponding metoprolol related compound in the *Standard solution*

C_S = concentration of the corresponding USP Metoprolol Related Compound RS in the *Standard solution* (µg/mL)

C_U = concentration of Metoprolol Fumarate in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified impurity in the portion of Metoprolol Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity in the *Sample solution*

r_S = peak response of metoprolol in the *Standard solution*

C_S = concentration of USP Metoprolol Fumarate RS in the *Standard solution* (µg/mL)

C_U = concentration of Metoprolol Fumarate in the *Sample solution* (µg/mL)

Acceptance criteria: See Table 2. Disregard peaks below 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fumaric acid	0.2	—
Metoprolol related compound C ^a	0.6	0.10
Metoprolol related compound B ^b	0.7	0.10
Metoprolol related compound A ^c	0.8	0.10
Metoprolol	1.0	—
Metoprolol related compound D ^d	1.5	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

^a 4-[2-Hydroxy-3-(isopropylamino)propoxy]benzaldehyde hydrochloride.

^b 1-Chloro-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.

^c 1-Ethylamino-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.

^d N,N-Bis(2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl)isopropylamine hydrochloride.

▲USP40

SPECIFIC TESTS

Delete the following:

- ▲ **MELTING RANGE OR TEMPERATURE** (741): 145°–148° ▲^{USP40}
- **PH** (791): 5.5–6.5, in a solution (1 in 10)
- **LOSS ON DRYING** (731)
Analysis: Dry under vacuum at 60° for 4 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

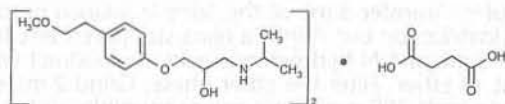
Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. ▲Store at controlled room temperature. ▲^{USP40}

Change to read:

- **USP REFERENCE STANDARDS** (11)
USP Metoprolol Fumarate RS
▲USP Metoprolol Related Compound A RS
1-Ethylamino-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.
 $C_{14}H_{23}NO_3$ 253.34
USP Metoprolol Related Compound B RS
1-Chloro-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.
 $C_{12}H_{17}ClO_3$ 244.71
USP Metoprolol Related Compound C RS
4-[2-Hydroxy-3-(isopropylamino)propoxy]benzaldehyde hydrochloride.
 $C_{13}H_{19}NO_3 \cdot HCl$ 273.76
USP Metoprolol Related Compound D RS
N,N-Bis[2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl]isopropylamine hydrochloride.
 $C_{27}H_{41}NO_6 \cdot HCl$ 512.08 ▲^{USP40}

Metoprolol Succinate



- $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$ 652.82
2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, (±)-, butanedioate (2:1) (salt).
(±)-1-(Isopropylamino)-3-[p-(2-methoxyethyl)phenoxy]-2-propanol succinate (2:1) (salt) [98418-47-4].

» Metoprolol Succinate contains not less than 98.0 percent and not more than 102.0 percent of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers at controlled room temperature.

USP Reference standards (11)—

- USP Metoprolol Related Compound A RS
(±)-1-Ethylamino-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.
 $C_{14}H_{23}NO_3$ 253.34
- USP Metoprolol Related Compound B RS
(±)-1-Chloro-2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propane.
 $C_{12}H_{17}ClO_3$ 244.71
- USP Metoprolol Related Compound C RS

(±)-4-[2-Hydroxy-3-(1-methylethyl)aminopropoxy]benzaldehyde.

$C_{13}H_{19}NO_3$ 237.29

USP Metoprolol Related Compound D RS

(±) N,N-Bis[2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl](1-methylethyl)amine.
 $C_{27}H_{41}NO_6$ 475.62

USP Metoprolol Succinate RS

Clarity and color of solution—A solution of Metoprolol Succinate having a concentration of 20 mg per mL is not less clear than an equal volume of water in a test tube of similar size. The absorbance of the solution determined at 440 nm in a 5-cm cell, using water as the blank, is not more than 0.1.

Identification, Infrared Absorption (197K).

PH (791): between 7.0 and 7.6, in a solution containing 65 mg per mL.

Loss on drying (731)—Dry it in vacuum at 60° for 4 hours; it loses not more than 0.2% of its weight.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals, Method I** (231): 0.001%. ● (Official 1-Jan-2018)

Related compounds—

TEST 1—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Test solution—Dissolve an accurately weighed quantity of Metoprolol Succinate in methanol to obtain a solution containing 50 mg per mL.

Standard solution—Dilute the *Test solution* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a concentration of 0.1 mg per mL.

Application volume: 10 µL.

Developing solvent system: a mixture of ethyl acetate and methanol (80:20).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Place two 50-mL beakers, each containing 30 mL of ammonium hydroxide, on the bottom of a chromatographic chamber that is lined with filter paper and contains the *Developing solvent system*, and allow to equilibrate for 1 hour. Position the plate in the chromatographic chamber, and develop the chromatogram until the solvent front has moved about two-thirds of the length of the plate. Remove the plate from the chamber, mark the solvent front, and dry the plate for 3 hours in a current of warm air. Place the plate in a chamber containing iodine vapor, and allow to react for at least 15 hours. Compare the intensities of the brown spots appearing on the chromatogram: any secondary spot obtained from the *Test solution* is not more intense than the corresponding spot obtained from the *Standard solution*. Not more than 0.2% is found.

TEST 2—

Sodium dodecyl sulfate solution, Mobile phase, and Resolution solution—Prepare as directed in the *Assay*.

Standard solution—Dissolve an accurately weighed quantity of USP Metoprolol Succinate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0 µg per mL.

Test solution—Transfer about 50 mg of Metoprolol Succinate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—Prepare as directed in the *Assay*. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between metoprolol related compound A and metoprolol related compound B is not less

than 2.5; and the resolution, R , between metoprolol related compound B and metoprolol related compound C is not less than 1.5. [NOTE—The relative retention times are about 0.6 for metoprolol related compound C, 0.7 for metoprolol related compound B, 0.8 for metoprolol related compound A, 1.0 for metoprolol, and 5.0 and 5.2 for the two diastereomers of metoprolol related compound D.] Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Inject equal volumes (about 10 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Metoprolol Succinate taken by the formula:

$$100(C_5 / C_1)(r_1 / r_3)$$

in which C_5 is the concentration, in mg per mL, of USP Metoprolol Succinate RS in the *Standard solution*; C_1 is the concentration of metoprolol succinate in the *Test solution*; r_1 is the individual peak response of related impurities; and r_3 is the peak response obtained from the *Standard solution*: not more than 0.1% of any single impurity is found, and not more than 0.5% of total impurities is found. [NOTE—The sum of the peak responses for the two diastereomers of metoprolol related compound D is used in the above calculation to report the amount of metoprolol related compound D.]

Assay—

Sodium dodecyl sulfate solution—Add 1.3 g of sodium dodecyl sulfate to 1 L of aqueous phosphoric acid, 0.1% (w/v).

Mobile phase—Prepare a filtered and degassed mixture of Sodium dodecyl sulfate solution and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Resolution solution—Prepare a solution in *Mobile phase* containing about 5 μ g each of USP Metoprolol Succinate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, USP Metoprolol Related Compound C RS, and USP Metoprolol Related Compound D RS per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Metoprolol Succinate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.08 mg per mL.

Test preparation—Transfer about 80 mg of Metoprolol Succinate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 223-nm detector and a 4-mm \times 12.5-cm column that contains 4- μ m packing L7. The column temperature is maintained at 30°. The flow rate is about 0.9 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, R , between metoprolol related compound A and metoprolol related compound B is not less than 2.5; and the resolution, R , between metoprolol related compound B and metoprolol related compound C is not less than 1.5. [NOTE—The relative retention times are about 0.6 for metoprolol related compound C, 0.7 for metoprolol related compound B, 0.8 for metoprolol related compound A, 1.0 for metoprolol, and 5.0 and 5.2 for the two diastereomers of metoprolol related compound D.] Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Test preparation* into the chro-

matograph, record the chromatograms for at least 1.5 times the retention of the metoprolol peak, and measure the peak responses. Calculate the quantity, in mg, of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$ in the portion of Metoprolol Succinate taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Metoprolol Succinate RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively.

Metoprolol Succinate Extended-Release Tablets

DEFINITION

Metoprolol Succinate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$.

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

Sample solution: Equivalent to 200 mg of metoprolol succinate from 1 or more Tablets to a stoppered centrifuge tube. Add 40 mL of pH 6.8 Phosphate Buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*) and 40 mL of methylene chloride, and shake for 5 min. Centrifuge, filter, and use the aqueous phase as the *Sample solution*.

Sample: Transfer 3 mL of the *Sample solution* to a separator. Add 2 mL of ammonium hydroxide, and extract with 20 mL of methylene chloride. Filter the methylene chloride phase. Grind 1 mL of the filtrate with 300 mg of potassium bromide, dry in a current of warm air, and prepare a disk.

Acceptance criteria: The IR spectrum of the *Sample* exhibits maxima only at the same wavelengths as that obtained from a similar preparation of USP Metoprolol Succinate RS (presence of metoprolol).

• B. INFRARED ABSORPTION (197K)

Sample: Transfer 5 mL of the *Sample solution* prepared in *Identification* test A into a glass-stoppered test tube. Add 2 mL of 5 N hydrochloric acid, and extract with 5 mL of ether. Filter the ether phase. Grind 2 mL of the filtrate with 300 mg of potassium bromide, dry in a current of warm air, and prepare a disk.

Acceptance criteria: The IR spectrum of the *Sample* exhibits maxima only at the same wavelengths as that obtained from a similar preparation of succinic acid (presence of succinate).

ASSAY

• PROCEDURE

Analysis: Determine the mean percentage value of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ from the Tablets analyzed in the test for *Uniformity of Dosage Units* (905).

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Medium: pH 6.8 Phosphate Buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 500 mL

Apparatus 2: 50 rpm

Times: 1, 4, 8, and 20 h

Buffer, Mobile phase, and Standard solution: Proceed as directed in the test for *Uniformity of Dosage Units* (905).

Analysis: Proceed as directed in the test for *Uniformity of Dosage Units* (905), except use 5.0 mL of a filtered

portion of the solution under test as the *Sample solution*, and use *Medium* as the blank, in comparison with a *Standard solution* having a known concentration of USP Metoprolol Succinate RS in the same *Medium*.
Acceptance criteria: See Table 1.

Table 1

Time (h)	Amount Dissolved (%)
1	NMT 25
4	20–40
8	40–60
20	NLT 80

The percentages of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* (711).

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: Simulated gastric fluid without enzyme, pH 1.2; 500 mL

Apparatus 2: 75 rpm

Time: 1, 4, 8, and 20 h

Buffer: 1 M monobasic sodium phosphate, 1 M phosphoric acid, and water (50:8:942). If necessary, adjust with 1 M monobasic sodium phosphate or 1 M phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (250:750)

Standard solution: Prepare a solution of USP Metoprolol Succinate RS in *Medium* as directed in *Table 2*.

Table 2

Tablet Strength (mg as metoprolol succinate)	Concentration (mg/mL)
200	0.380
100	0.190
50	0.095
25	0.048

Sample solution: Pass the solution under test through a suitable filter.

Chromatographic system
 (See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm \times 12.5-cm; 4- μ m packing L7

Flow rate: 1 mL/min

Injection volume: See *Table 3*.

Table 3

Tablet Strength (mg as metoprolol succinate)	Volume (μ L)
25	40
50	20
100	10
200	5

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis:

Samples: *Standard solution* and *Sample solution*

Calculate the concentration, C_i , in mg/mL, of metoprolol succinate dissolved in *Medium* at each time point, i :

$$\text{Result} = (r_u/r_s) \times C_s$$

r_u = peak response of metoprolol from the *Sample solution*

r_s = peak response of metoprolol from the *Standard solution*

C_s = concentration of USP Metoprolol Succinate RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ dissolved (Q_i), at each time point i :

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_5)] + [C_1 \times V_5]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times (V - (2 \times V_5))] + [(C_2 + C_1) \times V_5]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times (V - (3 \times V_5))] + [(C_3 + C_2 + C_1) \times V_5]\} \times (1/L) \times 100$$

C_i = concentration of metoprolol succinate in the portion of sample withdrawn at time point i (mg/mL)

V = volume of *Medium*; 500 mL

V_5 = volume of the *Sample solution* withdrawn from the *Medium* (mL)

L = label claim (mg/Tablet)

Tolerances: See *Table 4*.

Table 4

Time Point (i)	Time (hr)	Amount Dissolved (%)
1	1	NMT 20
2	4	20–40
3	8	55–85
4	20	NLT 80

The percentages of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* (711).

• UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

Procedure for content uniformity

Buffer: Mix 50 mL of 1 M monobasic sodium phosphate and 8.0 mL of 1 M phosphoric acid, and dilute with water to 1000 mL. If necessary, adjust with 1 M monobasic potassium phosphate or 1 M phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (250:750)

Standard solution: 0.05 mg/mL of USP Metoprolol Succinate RS in *Mobile phase*

Sample stock solution: Nominally 1 mg/mL of metoprolol succinate prepared as follows. Transfer 1 Tablet to a suitable volumetric flask, add about 5 mL of water, and allow the Tablet to disintegrate. Add a vol-

ume of alcohol to fill 30% of final volume, and shake for 30 min. Add a portion of 0.1 N hydrochloric acid to fill 50% of the flask volume, and shake for an additional 30 min. Dilute with 0.1 N hydrochloric acid to volume. Filter, and discard the first 10 mL of the filtrate.

Sample solution: Nominally 0.05 mg/mL of metoprolol succinate from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4-mm × 12.5-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 40 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metoprolol succinate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4]$ in the Tablet taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of metoprolol from the *Sample solution*

r_s = peak response of metoprolol from the *Standard solution*

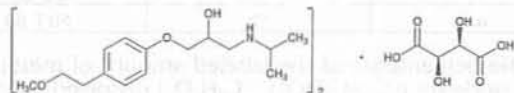
C_s = concentration of USP Metoprolol Succinate RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of metoprolol succinate in the *Sample solution* (mg/mL)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label it to indicate the content of metoprolol succinate and its equivalent, expressed as metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
USP Metoprolol Succinate RS

Metoprolol Tartrate



$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ 684.81

2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, (±)-, [R-(R*,R*)]-2,3-dihydroxybutanedioate (2:1) (salt);
(±)-1-(Isopropylamino)-3-[p-(2-methoxyethyl)phenoxy]-2-propanol 1-(+)-tartrate (2:1) (salt);
1-(Isopropylamino)-3-[p-(2-methoxyethyl)phenoxy]-2-propanol (2:1) *dextro*-tartrate salt [56392-17-7].

DEFINITION

Metoprolol Tartrate contains NLT 98.0% and NMT 102.0% of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

[NOTE—Use all solutions within 48 h.]

Buffer: 1.3 g/L of sodium dodecyl sulfate in 0.1% (w/v) phosphoric acid

Mobile phase: Acetonitrile and *Buffer* (400:600)

Standard solution: 1 mg/mL of USP Metoprolol Tartrate RS in *Mobile phase*

Sample solution: 1 mg/mL of Metoprolol Tartrate in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 223 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ in the portion of sample taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of metoprolol from the *Sample solution*

r_s = peak response of metoprolol from the *Standard solution*

C_s = concentration of USP Metoprolol Tartrate RS in the *Standard solution* (mg/mL)

C_u = concentration of Metoprolol Tartrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

Delete the following:

- **HEAVY METALS, Method I (231):** NMT 10 ppm • (Official 1-Jan-2018)

ORGANIC IMPURITIES

[NOTE—Use all solutions within 48 hrs.]

Buffer, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 5 µg/mL each of USP Metoprolol Tartrate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, and USP Metoprolol Related Compound C RS in *Mobile phase*

Standard solution: 1 µg/mL each of USP Metoprolol Tartrate RS, USP Metoprolol Related Compound A RS, USP Metoprolol Related Compound B RS, USP Metoprolol Related Compound C RS, and USP Metoprolol Related Compound D RS in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between metoprolol related compound A and metoprolol related compound B; NLT 2.5 between metoprolol related compound B

and metoprolol related compound C, *System suitability solution*

Relative standard deviation: NMT 5.0% for the metoprolol peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*.

Calculate the percentage of each impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of each individual impurity in the *Sample solution*
 r_S = peak response of the corresponding metoprolol related compound or metoprolol (for calculating any individual unspecified impurity) in the *Standard solution*
 C_S = concentration of the corresponding USP Metoprolol Related Compound RS or USP Metoprolol Tartrate RS (for calculating any individual unspecified impurity) in the *Standard solution* (mg/mL)
 C_U = concentration of Metoprolol Tartrate in the *Sample solution* (mg/mL)
Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Metoprolol related compound C ^a	0.65	0.10
Metoprolol related compound B ^b	0.72	0.10
Metoprolol related compound A ^c	0.83	0.10
Metoprolol	1.00	—
Metoprolol related compound D ^{d,e}	4.78/5.00	0.10
Any individual unspecified impurity	—	0.10
Total impurities ^f	—	0.50

^a (±)-4-[2-Hydroxy-3-(1-isopropyl)aminopropoxy]benzaldehyde.

^b (±)-1-Chloro-2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]-propane.

^c (±)-1-(Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol.

^d (±)-N,N-bis-[2-Hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl](1-methylethyl)amine hydrochloride.

^e The sum of the peak responses of the two diastereomers is used to calculate the amount of metoprolol related compound D.

^f Disregard any peak due to tartaric acid at about RRT 0.17.

SPECIFIC TESTS

• OPTICAL ROTATION, *Specific Rotation* (7815)

Sample solution: 20 mg/mL in water

Acceptance criteria: +6.5° to +10.5° (t = 20°)

• pH (791)

Sample solution: 100 mg/mL of Metoprolol Tartrate in water

Acceptance criteria: 6.0–7.0

• LOSS ON DRYING (731)

Sample solution: Dry a sample in a vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Metoprolol Tartrate RS

USP Metoprolol Related Compound A RS

(±)-1-(Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol.

C₁₄H₂₃NO₃ 253.34

USP Metoprolol Related Compound B RS

(±)-1-Chloro-2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]-propane.

C₁₂H₁₇ClO₃ 244.71

USP Metoprolol Related Compound C RS

(±)-4-[2-Hydroxy-3-(1-isopropyl)aminopropoxy]benzaldehyde.

C₁₃H₁₉NO₃ 237.29

USP Metoprolol Related Compound D RS

(±)-N,N-bis-[2-Hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl](1-methylethyl)amine hydrochloride.

C₂₇H₄₁NO₆ 512.08

Metoprolol Tartrate Injection

» Metoprolol Tartrate Injection is a sterile solution of Metoprolol Tartrate in Water for Injection. It contains Sodium Chloride as a tonicity-adjusting agent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of metoprolol tartrate [(C₁₅H₂₅NO₃)₂ · C₄H₆O₆].

Packaging and storage—Preserve in single-dose, light-resistant containers, preferably of Type I or Type II glass.

USP Reference standards (11)—

USP Endotoxin RS

USP Metoprolol Tartrate RS

USP Oxprenolol Hydrochloride RS

Identification—Place a volume of Injection, equivalent to about 40 mg of metoprolol tartrate, in a separator, add 4 mL of dilute ammonium hydroxide (1 in 3), and extract with 20 mL of chloroform, filtering the chloroform extract through chloroform-pre-rinsed anhydrous sodium sulfate. Evaporate the chloroform to dryness, and place in a freezer to congeal the residue: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Metoprolol Tartrate RS.

Bacterial Endotoxins Test (85)—It contains not more than 25.0 USP Endotoxin Units per mg of metoprolol tartrate.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

pH (791): between 5.0 and 8.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Mobile phase—Prepare a degassed solution by dissolving 961 mg of 1-pentanesulfonic acid sodium salt (monohydrate) and 82 mg of anhydrous sodium acetate in a mixture of 550 mL of methanol and 470 mL of water and adding 0.57 mL of glacial acetic acid.

Internal standard solution—Dissolve USP Oxprenolol Hydrochloride RS in freshly prepared *Mobile phase* to obtain a solution containing about 720 µg per mL.

Sodium chloride solution—Dissolve 9.0 g of sodium chloride in water to make 1000 mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Metoprolol Tartrate RS in *Sodium chloride solution* to obtain a stock solution having a known concentration of about 1000 µg per mL. Mix equal volumes, accurately measured, of this stock solution and of *Internal standard solution*.

Assay preparation—Dilute an accurately measured volume of Injection, if necessary, quantitatively with *Sodium chloride solution* to obtain a stock solution having a concentration of about 1000 µg per mL. Mix equal volumes, accurately measured, of this stock solution and of *Internal standard solution*.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph three replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*; the relative standard deviation is not more than 2.0%, and the resolution factor between metoprolol tartrate and oxprenolol hydrochloride is not less than 2.0.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.8 for metoprolol tartrate and 1.0 for oxprenolol hydrochloride. Calculate the quantity, in mg, of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ in each mL of the Injection taken by the formula:

$$(L/D)(C)(R_U/R_S)$$

in which *L* is the labeled quantity, in mg, of metoprolol tartrate in the Injection; *D* is the concentration, in µg per mL, of metoprolol tartrate in the *Assay preparation*, on the basis of the labeled quantity in each mL of Injection taken and the extent of dilution; *C* is the concentration, in µg per mL, of USP Metoprolol Tartrate RS in the *Standard preparation*; and *R_U* and *R_S* are the peak response ratios of metoprolol tartrate to oxprenolol hydrochloride obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Metoprolol Tartrate Compounded Oral Solution

DEFINITION

Metoprolol Tartrate Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$.

Prepare Metoprolol Tartrate Compounded Oral Solution 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Metoprolol Tartrate powder	1 g
Vehicle for Oral Solution (regular or sugar-free), <i>NF</i> , a sufficient quantity to make	100 mL

Add *Metoprolol Tartrate powder* and 20 mL of *Vehicle* to a mortar, and mix. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

ASSAY

PROCEDURE

Mobile phase: 961 mg of 1-pentanesulfonic acid sodium salt (monohydrate) and 82 mg of anhydrous sodium acetate in a mixture of 550 mL of methanol and 470 mL of water. Add 0.57 mL of glacial acetic acid. Filter, and degas.

Standard solution: 100 µg/mL of USP Metoprolol Tartrate RS

Sample solution: Agitate the container of Oral Solution for 30 min on a rotating mixer, remove a 5-mL sample,

and store in a clear glass vial at −70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix on a vortex mixer for 30 s. Pipet 1.0 mL of the sample to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for metoprolol tartrate is about 7.3 min.]

Suitability requirements

Relative standard deviation: NMT 1.3% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Metoprolol Tartrate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of metoprolol tartrate in the *Sample solution* (µg/mL)
Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **PH (791):** 3.6–4.6

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator
- **LABELING:** Label it to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS (11)**
USP Metoprolol Tartrate RS

Metoprolol Tartrate Compounded Oral Suspension

DEFINITION

Metoprolol Tartrate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$.

Prepare Metoprolol Tartrate Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Metoprolol Tartrate	1 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, (regular or sugar-free), <i>NF</i> , and Vehicle for Oral Suspension, <i>NF</i> , a sufficient quantity to make	100 mL

Place the required number of tablets in a suitable mortar, and comminute to a fine powder, or use *Metoprolol Tartrate powder*. Add the *Vehicle* in small portions, and mix

well. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add the *Vehicle* in portions to rinse the mortar. Add sufficient *Vehicle* to bring to final volume, and mix well.

ASSAY

PROCEDURE

Mobile phase: 961 mg of 1-pentanesulfonic acid sodium salt (monohydrate) and 82 mg of anhydrous sodium acetate in a mixture of 550 mL of methanol and 470 mL of water. Add 0.57 mL of glacial acetic acid. Filter, and degas.

Standard solution: 100 µg/mL of USP Metoprolol Tartrate RS

Sample solution: Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at -70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for metoprolol tartrate is about 7.3 min.]

Suitability requirements

Relative standard deviation: NMT 1.3% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metoprolol tartrate [(C₁₅H₂₅NO₃)₂ · C₄H₆O₆] in the portion of Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Metoprolol Tartrate RS in the *Standard solution* (µg/mL)

C_u = nominal concentration of metoprolol tartrate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **PH** (791): 3.6–4.6

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature, or in a refrigerator
- **LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** (11)
USP Metoprolol Tartrate RS

Metoprolol Tartrate Tablets

» Metoprolol Tartrate Tablets contain not less than 90.0 percent and not more than 110.0 per-

cent of the labeled amount of metoprolol tartrate [(C₁₅H₂₅NO₃)₂ · C₄H₆O₆].

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Metoprolol Tartrate RS

USP Oxprenolol Hydrochloride RS

Identification—

A: Place a quantity of finely powdered Tablets, equivalent to about 40 mg of metoprolol tartrate, in a separator, add 25 mL of water and 4 mL of dilute ammonium hydroxide (1 in 3), and extract with 20 mL of chloroform, filtering the chloroform extract through chloroform-pretreated anhydrous sodium sulfate. Evaporate the chloroform to dryness, and place in a freezer to congeal the residue: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Metoprolol Tartrate RS.

B: Transfer a portion of finely powdered Tablets, equivalent to about 50 mg of metoprolol tartrate, to a 500-mL volumetric flask, dilute with water to volume, and mix. Pass a portion of this solution through a filter of 1 µm or finer porosity: the UV spectrum of the filtrate exhibits maxima and minima at the same wavelengths as that of a solution of USP Metoprolol Tartrate RS in water having a concentration of about 0.1 mg per mL.

C: The retention time of the metoprolol peak in the chromatogram obtained from the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

Dissolution (711)—

Medium: simulated gastric fluid TS (without enzyme); 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of (C₁₅H₂₅NO₃)₂ · C₄H₆O₆ dissolved in filtered portions of the solution under test from UV absorbances at the wavelength of maximum absorbance at about 275 nm in comparison with a *Standard solution* having a known concentration of USP Metoprolol Tartrate RS in the same medium.

Tolerances—Not less than 75% (Q) of the labeled amount of (C₁₅H₂₅NO₃)₂ · C₄H₆O₆ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Solvent mixture—Prepare a mixture of methanol and 0.1 N hydrochloric acid (1:1).

Mobile phase—Prepare a suitable and degassed solution by dissolving 961 mg of 1-pentanesulfonic acid sodium salt (monohydrate) and 82 mg of anhydrous sodium acetate in a mixture of 550 mL of methanol and 470 mL of water and adding 0.57 mL of glacial acetic acid.

Standard preparation—Dissolve an accurately weighed quantity of USP Metoprolol Tartrate RS in *Solvent mixture* to obtain a stock solution having a known concentration of about 1000 µg per mL. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Resolution solution—Prepare a solution of oxprenolol hydrochloride in *Solvent mixture* to obtain a solution containing about 720 µg per mL. Prepare a 1:1 mixture of this solution and the stock solution used to prepare the *Standard preparation*.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of metoprolol tartrate, to a 50-mL volumetric flask, add 30 mL of *Solvent mix-*

ture, shake by mechanical means for 30 minutes, sonicate for 15 minutes, and heat on a steam bath for 10 minutes. Allow the solution to cool to room temperature, dilute with *Solvent mixture* to volume, and mix. Centrifuge a portion of the solution, and transfer 25.0 mL of the supernatant to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter of 0.5 μm or finer porosity, discarding the first few mL of the filtrate.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm \times 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.8 for metoprolol and 1.0 for oxprenolol, and the resolution, R , between the metoprolol and oxprenolol peaks is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 30 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of metoprolol tartrate $[(\text{C}_{15}\text{H}_{25}\text{NO}_3)_2 \cdot \text{C}_4\text{H}_6\text{O}_6]$ in the portion of Tablets taken by the formula:

$$0.1C(r_u / r_s)$$

in which C is the concentration, in μg per mL, of USP Metoprolol Tartrate RS in the *Standard preparation*; and r_u and r_s are the metoprolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Metoprolol Tartrate and Hydrochlorothiazide Tablets

» Metoprolol Tartrate and Hydrochlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of metoprolol tartrate $[(\text{C}_{15}\text{H}_{25}\text{NO}_3)_2 \cdot \text{C}_4\text{H}_6\text{O}_6]$ and hydrochlorothiazide $(\text{C}_7\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2)$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Benzothiadiazine Related Compound A RS
4-Amino-6-chloro-1,3-benzenedisulfonamide.
 $\text{C}_6\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$ 285.73
USP Hydrochlorothiazide RS
USP Metoprolol Tartrate RS
USP Oxprenolol Hydrochloride RS

Identification—

A: Place a quantity of finely powdered Tablets, equivalent to about 100 mg of metoprolol tartrate, in a 50-mL volumetric flask. Add about 30 mL of 0.1 N sodium hydroxide, shake for 20 minutes, dilute with 0.1 N sodium hydroxide to volume, and mix. Filter a portion of this mixture, discarding the first 10 mL of the filtrate. Place 25 mL of the filtrate into a separator, and extract with three 15-mL portions of chloroform, filtering the chloroform extracts through chloroform-prerinsed anhydrous sodium sulfate, and combining the extracts in a suitable container. [NOTE—Retain the aqueous layer remaining after extraction for *Identification test B.*] Evaporate the chloroform to dryness, and place in a freezer to congeal the residue: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained ex-

hibits maxima only at the same wavelengths as that of a similar preparation of USP Metoprolol Tartrate RS.

B: Pass the aqueous layer from *Identification test A* through 0.1 N sodium hydroxide-prerinsed cotton. Dilute a portion of the filtrate quantitatively and stepwise with 0.1 N sodium hydroxide to obtain a solution having a concentration of about 0.01 mg of hydrochlorothiazide per mL: the UV absorption spectrum of this solution exhibits maxima and minima at the same wavelengths as a Standard solution prepared as follows. Dissolve 25 mg of USP Hydrochlorothiazide RS in 50 mL of 0.1 N sodium hydroxide in a separator, and extract with three 15-mL portions of chloroform. Discard the chloroform extracts, and pass the aqueous solution through 0.1 N sodium hydroxide-prerinsed cotton. Pipet 2 mL of the filtrate into a 100-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.

Dissolution (711)—

Medium: simulated gastric fluid TS (without enzyme); 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Determination of dissolved metoprolol tartrate—Remove about 125 mL of the solution under test, allow to cool to room temperature, and filter, discarding the first 25 mL of the filtrate. [NOTE—Retain about 30 mL of the remaining filtrate of the solution under test for the *Determination of dissolved hydrochlorothiazide*.] If necessary, quantitatively dilute a portion of the filtrate with fresh *Dissolution Medium* to obtain a solution having a concentration of about 0.05 mg of metoprolol tartrate per mL. Transfer to separate separators 50.0 mL of the filtrate, 50.0 mL of a Standard solution in *Dissolution Medium* having a known concentration of about 0.05 mg of USP Metoprolol Tartrate RS per mL, and 50.0 mL of *Dissolution Medium* to provide the blank. Add 10 mL of 2.5 N sodium hydroxide to each separator, and extract each with three 15-mL portions of chloroform, filtering the chloroform extracts through pledgets of chloroform-prerinsed glass wool into individual 50-mL volumetric flasks. Dilute the contents of each flask with chloroform to volume, and mix. Determine the absorbances of the solutions from the filtrate and the Standard solution in 2-cm cells at the wavelength of maximum absorbance at about 276 nm, with a suitable spectrophotometer, using the solution from the blank to set the instrument. Calculate the quantity, in mg, of $(\text{C}_{15}\text{H}_{25}\text{NO}_3)_2 \cdot \text{C}_4\text{H}_6\text{O}_6$ dissolved by the formula:

$$900Cf(A_u / A_s)$$

in which C is the concentration, in mg per mL, of USP Metoprolol Tartrate RS in the *Standard solution*; f is the dilution factor for the solution from the filtrate; and A_u and A_s are the absorbances of the solution from the filtrate and of the *Standard solution*, respectively.

Determination of dissolved hydrochlorothiazide—Filter a portion of the filtrate retained from the *Determination of dissolved metoprolol tartrate* through a filter of 0.8 μm or finer porosity, discarding the first 5 mL of the filtrate. If necessary, quantitatively dilute a portion of the filtrate with fresh *Dissolution Medium* to obtain a solution having a concentration of about 0.03 mg of hydrochlorothiazide per mL. Prepare a *Standard solution* in *Dissolution Medium* having a known concentration of about 0.03 mg of USP Hydrochlorothiazide RS per mL. Determine the absorbances of these solutions in 2-cm cells at the wavelength of maximum absorbance at about 316 nm, using *Dissolution Medium* as the blank. Calculate the quantity, in mg, of $\text{C}_7\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$ dissolved by the formula:

$$900Cf(A_u / A_s)$$

in which C is the concentration, in mg per mL, of USP Hydrochlorothiazide RS in the *Standard solution*; f is the dilu-

tion factor for the solution from the filtrate; and A_U and A_S are the absorbances of the solution from the filtrate and of the *Standard solution*, respectively.

Tolerances—Not less than 80% (Q) of the labeled amount of metoprolol tartrate ($C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ and not less than 80% (Q) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements for *Content Uniformity* with respect to metoprolol tartrate and to hydrochlorothiazide.

Procedure for content uniformity of metoprolol tartrate—Transfer 1 Tablet to a suitable volumetric flask, add 0.1 N hydrochloric acid to about 60% of the nominal volume, sonicate for 15 minutes, and shake by mechanical means for 30 minutes. Dilute with 0.1 N hydrochloric acid to volume to obtain a final solution having a concentration of approximately 1000 μ g per mL. Mix, and filter, discarding the first 20 mL of the filtrate. Pipet 10 mL of the filtrate, 10 mL of a *Standard solution* of USP Metoprolol Tartrate RS in the same medium having a known concentration of about 1000 μ g per mL, and 10 mL of 0.1 N hydrochloric acid to provide a blank into individual separators. To each separator add 2.0 mL of 2.5 N sodium hydroxide, and extract with three 25-mL portions of chloroform, passing the chloroform extracts through chloroform-pre-rinsed glass wool into individual 100-mL volumetric flasks. Dilute with chloroform to volume, and mix. Determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 276 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of ($C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ in the Tablet by the formula:

$$(T/1000)(A_U / A_S)$$

in which T is the labeled quantity, in mg, of metoprolol tartrate in the Tablet; C is the concentration, in μ g per mL, of USP Metoprolol Tartrate RS in the *Standard solution*; and A_U and A_S are the absorbances of the solution from the Tablet and the *Standard solution*, respectively.

Procedure for content uniformity of hydrochlorothiazide—Transfer 1 Tablet to a 100-mL volumetric flask containing 15 mL of water, and shake by mechanical means for 15 minutes. Add 60 mL of methanol, sonicate for 5 minutes, and shake by mechanical means for 30 minutes. Dilute with methanol to volume, and mix. Centrifuge 40 mL of this suspension. Dilute an accurately measured portion of the supernatant quantitatively with methanol to obtain a solution having a concentration of about 0.05 mg of hydrochlorothiazide per mL. Filter a portion of this solution through a 0.5- μ m porosity filter, discarding the first few mL of the filtrate. Use the filtrate as the test solution. Transfer about 25 mg of USP Hydrochlorothiazide RS, accurately weighed, to a 100-mL volumetric flask containing 15 mL of water, dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix (*Standard solution*). Concomitantly determine the absorbances of the test solution and the *Standard solution* at the wavelength of maximum absorbance at about 316 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the Tablet taken by the formula:

$$(LC/D)(A_U / A_S)$$

in which L is the labeled quantity, in mg, of hydrochlorothiazide in the Tablet; C is the concentration, in mg per mL, of USP Hydrochlorothiazide RS in the *Standard solution*; D is the concentration, in mg per mL, of hydrochlorothiazide in the test solution based on the labeled quantity in the Tablet and the extent of dilution; and A_U and A_S are the absorbances of the *Test solution* and the *Standard solution*, respectively.

Diazotizable substances—

Standard solution—Weigh accurately 5 mg of USP Benzothiadiazine Related Compound A RS, and dissolve in 2 mL of methanol contained in a 50-mL volumetric flask. Dilute with water to volume, and mix. Pipet 5 mL of the resulting solution into a 100-mL volumetric flask, add 20 mL of methanol, dilute with water to volume, and mix. Each mL of *Standard solution* contains 5 μ g of the Reference Standard.

Test solution—Transfer a portion of the powdered Tablets prepared for the Assay, accurately weighed and equivalent to 50 mg of hydrochlorothiazide, to a 100-mL volumetric flask containing a mixture of 20 mL of methanol and 20 mL of water. Shake continuously for 5 to 10 minutes, dilute with water to volume, mix, and filter. Use the filtrate as the *Test solution* immediately after preparation.

Procedure—Pipet 5 mL each of the *Standard solution* and the *Test solution* into separate, 50-mL volumetric flasks. Pipet 5 mL of water into a third 50-mL volumetric flask to provide a blank. To each flask add 1 mL of freshly prepared sodium nitrite solution (1 in 100) and 5 mL of dilute hydrochloric acid (1 in 10), and allow to stand for 5 minutes. Add 2 mL of ammonium sulfamate solution (1 in 50), allow to stand for 5 minutes with frequent swirling, then add 2 mL of freshly prepared disodium chromotropate solution (1 in 100) and 10 mL of sodium acetate TS. Dilute with water to volume, and mix. Concomitantly determine the absorbances of the solutions obtained from the *Standard solution* and the *Test solution* at 500 nm, with a suitable spectrophotometer, against the blank. The absorbance of the solution from the *Test solution* does not exceed that of the solution from the *Standard solution*, corresponding to not more than 1.0% of diazotizable substances.

Assay for metoprolol tartrate—

Mobile phase and Chromatographic system—Proceed as directed in the Assay under *Metoprolol Tartrate Injection*.

Internal standard solution—Dissolve USP Oxprenolol Hydrochloride RS in freshly prepared *Mobile phase* to obtain a solution containing about 360 μ g per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Metoprolol Tartrate RS in 0.1 N hydrochloric acid to obtain a stock solution having a known concentration of about 1000 μ g per mL. Transfer 10.0 mL of this stock solution to a suitable separator, add 2.0 mL of 2.5 N sodium hydroxide, and extract with three 25-mL portions of chloroform. Pass the chloroform extracts through chloroform-pre-rinsed glass wool into a round-bottom flask, and evaporate on a rotary evaporator under vacuum to dryness. Add 20.0 mL of *Internal standard solution* to the flask, sonicate for 3 minutes, and gently swirl to dissolve the residue in the flask.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of metoprolol tartrate, to a 100-mL volumetric flask, add 60 mL of 0.1 N hydrochloric acid, heat on a steam bath for 3 minutes, and sonicate for 5 minutes. Shake for 30 minutes. Allow the solution to cool to room temperature, dilute with 0.1 N hydrochloric acid to volume, and mix. Filter a portion of this solution, discarding the first 20 mL of the filtrate. Transfer 10.0 mL of the filtrate to a separator, add 2.0 mL of 2.5 N sodium hydroxide, and extract with three 25-mL portions of chloroform. Pass the chloroform extracts through chloroform-pre-rinsed glass wool into a round-bottom flask, and evaporate on a rotary evaporator under vacuum to dryness. Add 20.0 mL of *Internal standard solution* to the flask, sonicate for 3 minutes, and gently swirl to dissolve the residue in the flask. Filter a portion of this solution through a filter of 0.5 μ m or finer porosity, discarding the first few mL of the filtrate. Use the filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the Assay under *Metoprolol Tartrate Injection*. Calculate the quantity, in

mg, of metoprolol tartrate $[(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6]$ in the portion of Tablets taken by the formula:

$$0.1 C(R_U / R_S)$$

in which C is the concentration, in μg per mL, of USP Metoprolol Tartrate RS in the stock solution used to prepare the *Standard preparation*; and R_U and R_S are the peak response ratios of metoprolol tartrate to oxprenolol hydrochloride obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for hydrochlorothiazide—

Mobile phase—[NOTE—Pass the methanol and water through 0.5- μm porosity filters before use.] Dissolve 1.38 g of monobasic sodium phosphate in 780 mL of water, add 220 mL of methanol, and mix. Degas before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard solution—Dissolve a quantity of sulfanilamide in methanol to obtain a solution containing about 0.4 mg per mL.

System suitability solution—Dissolve a quantity of USP Benzothiadiazine Related Compound A RS in *Internal standard solution* to obtain a solution containing about 1 mg per mL. Mix 1 mL of this solution and 4 mL of methanol.

Standard preparation—Transfer about 50 mg of USP Hydrochlorothiazide RS, accurately weighed, to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 25 mg of hydrochlorothiazide, to a 50-mL volumetric flask. Add 10.0 mL of *Internal standard solution* and 20 mL of methanol, and sonicate for 5 minutes. Shake by mechanical means for 30 minutes, dilute with methanol to volume, and mix. Centrifuge a portion of this solution, and pass a portion of the supernatant through a 0.5- μm porosity filter, discarding the first few mL of the filtrate. Use the filtrate as the *Assay preparation*.

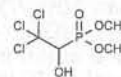
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm \times 30-cm column that contains packing L1. The flow rate is about 0.6 mL per minute. Chromatograph replicate injections of the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for sulfanilamide and 1.0 for benzothiadiazine related compound A; and the resolution, R , between the peaks is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 4 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for sulfanilamide and 1.0 for hydrochlorothiazide. Calculate the quantity, in mg, of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken by the formula:

$$50C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Hydrochlorothiazide RS in the *Standard preparation*; and R_U and R_S are the peak response ratios of hydrochlorothiazide to sulfanilamide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Metrifonate



$C_4H_8Cl_3O_4P$ 257.44

Phosphonic acid, (2,2,2-trichloro-1-hydroxyethyl)-, dimethyl ester.

Dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate [52-68-6].

» Metrifonate contains not less than 98.0 percent and not more than 100.5 percent of $C_4H_8Cl_3O_4P$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers at a temperature not exceeding 25°.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Metrifonate RS

Trichlorfon.

Completeness of solution (641): meets the requirements, 0.5 g of it being dissolved in methanol.

Color of solution (631)—The solution obtained in the test for *Completeness of solution* has no more color than *Matching Fluid F*.

Identification—

A: Infrared Absorption (197K).

B: Thin-Layer Chromatographic Identification Test (201)—

Test solution: Dissolve 10 mg of Metrifonate in methanol, and dilute with methanol to 10.0 mL.

Developing solvent system: a mixture of toluene, dioxane, and glacial acetic acid (70:25:5)

Procedure—Proceed as directed in the chapter. After allowing the plate to air-dry, spray the plate with a 5% solution of 4-(p-nitrobenzyl)pyridine in acetone, and heat at 120° for 15 minutes. Before the plate cools, spray it with a 10% solution of tetraethylenepentamine in acetone, and immediately examine the plate: the principal spot in the chromatogram obtained from the *Test solution* corresponds in R_f value, size, and blue color to that in the chromatogram obtained from the *Standard solution*.

C: Dissolve 20 mg of Metrifonate in 1 mL of 2 N sodium hydroxide, add 1 mL of pyridine, shake, and heat on a water bath for 2 minutes: a red color develops in the pyridine layer.

D: To 100 mg of Metrifonate add 0.5 mL of nitric acid, 0.5 mL of a 50% solution of ammonium nitrate, and 0.1 mL of 30 percent hydrogen peroxide, and heat on a water bath for 10 minutes. Heat to boiling, and add 1 mL of ammonium molybdate TS: a yellow color precipitate is formed.

Acidity—Dissolve 2.5 g of it in carbon dioxide-free water, dilute with carbon dioxide-free water to 50 mL, and add 0.1 mL of methyl red TS. Not more than 1.0 mL of 0.1 N sodium hydroxide is required to change the color of the indicator.

Water Determination, Method I (921): not more than 0.3%.

Delete the following:

• **Heavy metals** (231): 0.001%. (Official 1-Jan-2018)

Limit of free chloride—Dissolve 5.0 g of Metrifonate in 30 mL of alcohol, and add a mixture of 100 mL of water and 15 mL of nitric acid. Titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically using a silver

electrode. Not more than 0.7 mL of 0.1 N silver nitrate is consumed (0.05%).

Chromatographic purity—

Solution A—Dissolve 1.36 g of monobasic potassium phosphate in water, and dilute with water to 1000 mL. Adjust with phosphoric acid to a pH of 3.0.

Solution B—Use acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Diluent—Prepare a mixture of acetonitrile and water (1:1).

Standard preparation—Prepare a solution of USP Metrifonate RS in *Diluent* containing 20 mg per mL.

Test solution—Transfer 500 mg of Metrifonate, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4-mm × 25-cm column that contains 5-μm packing L7. The column is maintained at a constant temperature of about 40°. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	90	10	equilibration (10 minutes)
0–5	90	10	isocratic
5	90→85	10→15	step gradient
5–25	85	15	isocratic
25	85→45	15→55	step gradient
25–end*	45	55	isocratic

*The elution concludes at 3 times the retention time of metrifonate.

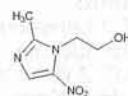
Procedure—Separately inject equal volumes (about 50 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of each impurity taken by the formula:

$$100F(r_i / r_s)$$

in which *F* is a response factor, being 0.38 for the desmethylmetrifonate peak, if present at a retention time of 0.5 relative to that of Metrifonate, 0.03 for the dichlorvos peak, if present, at a retention time of 1.9 relative to that of Metrifonate, and 1.0 for any other impurity; *r_i* is the peak area for the individual impurity obtained from the *Test solution*; and *r_s* is the peak area for Metrifonate obtained from the *Standard solution*: not more than 1.0% of desmethylmetrifonate, 0.2% of dichlorvos, and 0.5% of any other impurity are found; and a total of not more than 1.0% of impurities other than desmethylmetrifonate and dichlorvos is found.

Assay—Dissolve about 300 mg of Metrifonate, accurately weighed, in 30 mL of alcohol. Add 10 mL of monoethanolamine, and allow to stand for 1 hour at 21 ± 1°. Cool while adding a mixture of 100 mL of water and 15 mL of nitric acid. While maintaining the temperature at 21 ± 1°, titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically using a silver electrode. Each mL of 0.1 N silver nitrate is equivalent to 25.74 mg of C₄H₈Cl₃O₄P.

Metronidazole



C₆H₉N₃O₃

171.15

1*H*-Imidazole-1-ethanol, 2-methyl-5-nitro-;
2-Methyl-5-nitroimidazole-1-ethanol [443-48-1].

DEFINITION

Metronidazole contains NLT 99.0% and NMT 101.0% of metronidazole (C₆H₉N₃O₃), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (177K): Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Mobile phase: Methanol and water (1:4)

Standard solution: 0.03 mg/mL of USP Metronidazole RS in *Mobile phase*

Sample solution: 0.03 mg/mL of Metronidazole in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 319 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 30 μL

Run time: Twice the retention time of metronidazole

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of metronidazole (C₆H₉N₃O₃) in the portion of Metronidazole taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 50 ppm (Official 1-Jan-2018)

ORGANIC IMPURITIES

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*. The run time is 30 min.

Standard solution: 1 μg/mL of metronidazole from USP Metronidazole RS and 2 μg/mL of tinidazole related compound A from USP Tinidazole Related Compound A RS in *Mobile phase*

Sample solution: 1.0 mg/mL of Metronidazole in *Mobile phase*

System suitabilitySample: *Standard solution*[NOTE—See *Table 1* for the relative retention times.]**Suitability requirements****Resolution:** NLT 2.0 between tinidazole related compound A and metronidazole**Tailing factor:** NMT 2.0 for the metronidazole peak**Relative standard deviation:** NMT 6.0% for both tinidazole related compound A and metronidazole; six replicate injections**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tinidazole related compound A in the portion of Metronidazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of tinidazole related compound A from the *Sample solution* r_S = peak response of tinidazole related compound A from the *Standard solution* C_S = concentration of USP Tinidazole Related Compound A RS in the *Standard solution* (mg/mL) C_U = concentration of Metronidazole in the *Sample solution* (mg/mL)

Calculate the percentage of any single unspecified impurity in the portion of Metronidazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of any single unspecified impurity from the *Sample solution* r_S = peak response of metronidazole from the *Standard solution* C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL) C_U = concentration of Metronidazole in the *Sample solution* (mg/mL)**Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.75	0.1
Metronidazole	1.00	—
Any single unspecified impurity	—	0.1
Total impurities	—	0.2

SPECIFIC TESTS• **Loss on Drying (731)**

Analysis: Dry at 105° for 2 h.

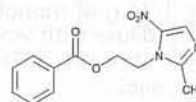
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.• **USP REFERENCE STANDARDS (11)**

USP Metronidazole RS

USP Tinidazole Related Compound A RS

2-Methyl-5-nitroimidazole.

C₄H₅N₃O₂ 127.10**Metronidazole Benzoate**C₁₃H₁₃N₃O₄

275.26

2-(2-Methyl-5-nitroimidazol-1-yl)ethyl benzoate
[13182-89-3].**DEFINITION**Metronidazole Benzoate contains NLT 98.5% and NMT 101.0% of metronidazole benzoate (C₁₃H₁₃N₃O₄), calculated on the dried basis.**IDENTIFICATION**• **A. INFRARED ABSORPTION (17K)**• **B.** The principal spot of the *Sample solution* corresponds to that of *Standard solution A*, as obtained in the test for *Organic Impurities*.**ASSAY**• **PROCEDURE****Sample solution:** Dissolve with stirring 250 mg of Metronidazole Benzoate in 50.0 mL of glacial acetic acid.**Titrimetric system**(See *Titrimetry* (541).)**Mode:** Direct titration**Titrant:** 0.1 N perchloric acid**Endpoint detection:** Potentiometric**Analysis:** Titrate the *Sample solution* with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.53 mg of metronidazole benzoate (C₁₃H₁₃N₃O₄).**Acceptance criteria:** 98.5%–101.0% on the dried basis**IMPURITIES**• **RESIDUE ON IGNITION (281):** NMT 0.1%**Delete the following:**• **HEAVY METALS, Method II (231):** NMT 20 ppm • (Official 1-Jan-2018)• **ORGANIC IMPURITIES****Standard solution A:** 0.1 mg/mL of USP Metronidazole Benzoate RS in acetone**Standard solution B:** 0.04 mg/mL of USP Metronidazole Benzoate RS in acetone from *Standard solution A***Standard solution C:** 0.2 mg/mL each of USP Metronidazole RS and USP Tinidazole Related Compound A RS in acetone**Sample solution:** 20 mg/mL of Metronidazole Benzoate in acetone**Chromatographic system**(See *Chromatography* (621), *Thin-Layer Chromatography*.)**Mode:** TLC**Adsorbent:** 0.2-mm layer of chromatographic silica gel mixture**Application volume:** 10 µL**Developing solvent system:** Ethyl acetate**System suitability****Sample:** *Standard solution C***Suitability requirements:** The test is valid only if the metronidazole and tinidazole related compound A spots are clearly separated.**Analysis****Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Proceed as directed in the chapter. Examine the plate under short-wavelength UV light.

Acceptance criteria: No secondary spot of the *Sample solution* is larger or more intense than the principal spot of *Standard solution A* (0.5%); and NMT three spots, excluding the principal spot, of the *Sample solution* are larger or more intense than the principal spot of *Standard solution B* (0.2%).

SPECIFIC TESTS

• ACIDITY

Sample: 2.0 g

Analysis: Neutralize 40 mL of a mixture of dimethylformamide and water (1:1) with hydrochloric acid or 0.02 M sodium hydroxide. Add 0.2 mL of methyl red TS and the *Sample*, mix to dissolve, and titrate with 0.02 M sodium hydroxide.

Acceptance criteria: NMT 0.25 mL is required to produce a color change.

• LOSS ON DRYING (731)

Analysis: Dry at 80° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

• USP REFERENCE STANDARDS (11)

USP Metronidazole RS

USP Metronidazole Benzoate RS

USP Tinidazole Related Compound A RS

2-Methyl-5-nitroimidazole.

C₄H₅N₃O₂ 127.10

Metronidazole Benzoate Compounded Oral Suspension

DEFINITION

Metronidazole Benzoate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole (C₆H₉N₃O₃).

Prepare Metronidazole Benzoate Compounded Oral Suspension containing 50 mg/mL of metronidazole as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Metronidazole (as the Benzoate) powder	5 g (8 g)
Ora-Blend ^a , a sufficient quantity to make	100 mL

^a Perrigo, Minneapolis, MN.

Place the *Metronidazole Benzoate powder* into a suitable mortar. Wet the powder with a small amount of *Ora-Blend*, and triturate to make a smooth paste. Add the *Ora-Blend* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated container. Add sufficient *Ora-Blend* to bring the preparation to final volume. Shake to mix well.

ASSAY

• PROCEDURE

Solution A: 0.1% (v/v) glacial acetic acid in water

Mobile phase: Acetonitrile and *Solution A* (40:60). Filter, and degas.

Standard solution: 0.4 mg/mL of metronidazole prepared from USP Metronidazole Benzoate RS in *Mobile phase*. Mix well until dissolved.

Sample solution: Shake thoroughly each bottle of Oral Suspension. Transfer 0.8 mL of the Oral Suspension into

a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix well.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 316 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for metronidazole is about 7.7 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metronidazole (C₆H₉N₃O₃) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of metronidazole in the *Standard solution* (mg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

SPECIFIC TESTS

• **pH (791):** 3.6–4.6

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or controlled room temperature.

• **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS (11)**

USP Metronidazole Benzoate RS

Metronidazole Capsules

DEFINITION

Metronidazole Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole (C₆H₉N₃O₃).

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

Wavelength range: Between 1600 and 1000 cm⁻¹

Acceptance criteria: Capsule contents show maxima only at the same wavelengths as those of similarly prepared USP Metronidazole RS.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Mobile phase: Methanol and water (1:4)

Standard solution: 0.03 mg/mL of USP Metronidazole RS in *Mobile phase*

Sample stock solution: Nominally 1 mg/mL of metronidazole prepared as follows. Mix the contents of

Capsules (NLT 20). Transfer an amount equivalent to 100 mg of metronidazole to a 100-mL volumetric flask, add 80 mL of *Mobile phase*, and sonicate with intermittent shaking for 10 min. Shake for 30 min, and dilute with *Mobile phase* to volume. Centrifuge a portion of the solution.

Sample solution: 0.03 mg/mL of metronidazole in *Mobile phase*, from the *Sample stock solution*. Pass a portion of the solution through a nylon membrane filter of 0.45- μ m or finer pore size. Discard the first 10 mL of the filtrate, and use the remainder.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 319 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 30 μ L

Run time: 2 times the retention time of the metronidazole peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for five replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: 0.4 mg/mL of USP Metronidazole RS in *Medium*. Sonicate to dissolve if necessary.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size, and discard the first few mL.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 278 nm

Cell: 0.05 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (1/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of *Medium*, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of metronidazole ($C_6H_9N_3O_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 1 μ g/mL of metronidazole from USP Metronidazole RS and 2 μ g/mL of tinidazole related compound A from USP Tinidazole Related Compound A RS in *Mobile phase*

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for tinidazole related compound A and metronidazole are 0.75 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between tinidazole related compound A and metronidazole

Tailing factor: NMT 2.0 for metronidazole

Relative standard deviation: NMT 6.0% for both tinidazole related compound A and metronidazole

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tinidazole related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tinidazole related compound A from the *Sample solution*

r_S = peak response of tinidazole related compound A from the *Standard solution*

C_S = concentration of USP Tinidazole Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any unspecified degradation product from the *Sample solution*

r_S = peak response of metronidazole from the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.75	0.1
Metronidazole	1.0	—
Each unspecified degradation product	—	0.1
Total impurities	—	0.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Metronidazole RS

USP Tinidazole Related Compound A RS

2-Methyl-5-nitroimidazole.

$C_4H_5N_3O_2$ 127.10

Metronidazole Gel

DEFINITION

Metronidazole Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$).

IDENTIFICATION

- **A.** The UV spectrum of the metronidazole peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: Methanol and water (20:80)

Solution B: Methanol

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10.0	100	0
15.0	10	90
15.1	100	0
20.0	100	0

System suitability solution: 0.6 µg/mL of USP Metronidazole RS and 0.6 µg/mL of USP Tinidazole Related Compound A RS in *Solution A*

Standard solution: 30 µg/mL of USP Metronidazole RS in *Solution A*

Sample stock solution: Nominally 300 µg/mL of metronidazole in *Solution A* prepared as follows. Transfer a portion of Gel to a suitable volumetric flask. Add *Solution A* equivalent to 50% of the flask volume and sonicate or vortex until dissolved. Dilute with *Solution A* to volume. [NOTE—On the basis of formulation, if necessary, centrifuge a portion of the solution at 3000 rpm for 10 min and pass a portion of the supernatant through a filter of 0.45-µm pore size. Use the filtrate.]

Sample solution: Nominally 30 µg/mL of metronidazole in *Solution A* prepared from the *Sample stock solution*

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 319 nm. For *Identification test A*, use a diode-array detector in the range of 210–500 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 30 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between metronidazole and tinidazole related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metronidazole from the *Sample solution*

r_S = peak response of metronidazole from the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• ORGANIC IMPURITIES

Solution A, Solution B, Mobile phase, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: Use the *System suitability solution* from the Assay.

Sample solution: Use the *Sample stock solution* from the Assay.

System suitability

Sample: *Standard solution*

[NOTE—See Table 2 for the relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between metronidazole and tinidazole related compound A

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tinidazole related compound A in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tinidazole related compound A from the *Sample solution*

r_S = peak response of tinidazole related compound A from the *Standard solution*

C_S = concentration of USP Tinidazole Related Compound A RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Calculate the percentage of each individual unspecified impurity in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response of metronidazole from the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.76	0.2
Metronidazole	1.0	—
Any individual unspecified impurity	—	0.3
Total impurities	—	1.0

PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

SPECIFIC TESTS

- **PH (791):** The apparent pH determined potentiometrically is between 4.0 and 6.5.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in laminated collapsible tubes at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
 - USP Metronidazole RS
 - USP Tinidazole Related Compound A RS
 - 2-Methyl-5-nitroimidazole.
 - $C_4H_5N_3O_2$ 127.10

Metronidazole Injection**DEFINITION**

Metronidazole Injection is a sterile, isotonic, buffered solution of Metronidazole in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole ($C_4H_5N_3O_2$).

IDENTIFICATION

- **A.** The UV (UV-Vis) spectrum of the metronidazole peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Mobile phase: Methanol and water (20:80)

System suitability solution: 1 µg/mL of USP Metronidazole RS and 2 µg/mL of USP Tinidazole Related Compound A RS in *Mobile phase*

Standard solution: 0.03 mg/mL of USP Metronidazole RS in *Mobile phase*

Sample solution: Nominally 0.03 mg/mL of metronidazole in *Mobile phase* prepared as follows. Transfer a portion of Injection to a suitable volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 319 nm. For *Identification test A*, use a diode array detector in the range of 210–800 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 30 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between metronidazole and tinidazole related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of metronidazole ($C_4H_5N_3O_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES**• ORGANIC IMPURITIES**

Mobile phase, Chromatographic system, and System suitability solution: Proceed as directed in the *Assay*.

Standard solution: 0.75 µg/mL each of USP Metronidazole RS and USP Tinidazole Related Compound A RS in *Mobile phase*

Sample solution: Nominally 500 µg/mL of metronidazole prepared as follows. Transfer a portion of Injection to a suitable volumetric flask. Add *Mobile phase* equivalent to 50% of the flask size. Sonicate for 2 min. Dilute with *Mobile phase* to volume, and pass a portion of the solution through a filter of 0.45-µm pore size. Use the filtrate.

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between metronidazole and tinidazole related compound A, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tinidazole related compound A in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tinidazole related compound A from the *Sample solution*

r_S = peak response of tinidazole related compound A from the *Standard solution*

C_S = concentration of USP Tinidazole Related Compound A RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each unspecified impurity from the *Sample solution*

r_S = peak response of metronidazole from the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.7	0.15
Metronidazole	1.0	—
Any individual unspecified degradation product	—	0.15
Total impurities	—	2.0

SPECIFIC TESTS

• **PH (791):** 4.5–7.0

• **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.35 USP Endotoxin Units/mg of metronidazole

- **PARTICULATE MATTER IN INJECTIONS** (788): It meets the requirements for small-volume injections.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers of Type I or Type II glass, or in suitable plastic containers, protected from light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
 - USP Endotoxin RS
 - USP Metronidazole RS
 - USP Tinidazole Related Compound A RS
 - 2-Methyl-5-nitroimidazole.
 - $C_4H_5N_3O_2$ 127.10

Metronidazole Tablets

DEFINITION

Metronidazole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$).

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION** (197U)
 - Sample stock solution:** Equivalent to 15 mg/mL of metronidazole from powdered Tablets in dilute hydrochloric acid (1 in 100). Shake for several min, and filter.
 - Medium:** Sulfuric acid in methanol (1 in 350)
 - Sample solution:** 20 µg/mL of metronidazole in *Medium* from the *Sample stock solution*
 - Acceptance criteria:** Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**
 - Mobile phase:** Methanol and water (20:80)
 - Standard solution:** 0.5 mg/mL of USP Metronidazole RS in *Mobile phase*
 - Sample stock solution:** Nominally 10 mg/mL of metronidazole in methanol from Tablets prepared as follows. Transfer 10 Tablets, whole or ground, to a suitable size volumetric flask. Add methanol, and shake by mechanical means for 30 min or until the Tablets are disintegrated. Dilute with methanol to volume, and allow the solution to stand until the insoluble material has settled.
 - Sample solution:** Nominally 0.5 mg/mL of metronidazole in *Mobile phase* prepared from the clear supernatant of the *Sample stock solution*. Filter.
- Chromatographic system**
 - (See *Chromatography* (621), *System Suitability*.)
 - Mode:** LC
 - Detector:** UV 254 nm
 - Column:** 4.6-mm × 15-cm; packing L7
 - Flow rate:** 1 mL/min
 - Injection volume:** 10 µL
- System suitability**
 - Sample:** *Standard solution*
 - Suitability requirements**
 - Tailing factor:** NMT 2
 - Relative standard deviation:** NMT 2.0%
- Analysis**
 - Samples:** *Standard solution* and *Sample solution*
 - Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of metronidazole from the *Sample solution*
- r_S = peak response of metronidazole from the *Standard solution*
- C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)
- C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)
- Acceptance criteria:** 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** (711)
 - Medium:** 0.1 N hydrochloric acid; 900 mL
 - Apparatus 1:** 100 rpm
 - Time:** 60 min
 - Standard solution:** USP Metronidazole RS in *Medium*
 - Sample solution:** Filter a portion of the solution under test, and dilute with *Medium* to a concentration similar to that of the *Standard solution*.
- Instrumental conditions**
 - Mode:** UV
 - Analytical wavelength:** 278 nm
 - Blank:** *Medium*
- Analysis**
 - Samples:** *Standard solution*, *Sample solution*, and *Blank*
 - Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) dissolved.

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times D \times 100$$

- A_U = absorbance of the *Sample solution*
- A_S = absorbance of the *Standard solution*
- C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL)
- V = volume of *Medium*, 900 mL
- L = label claim (mg/Tablet)
- D = dilution factor to prepare the *Sample solution*
- Tolerances:** NLT 85% (Q) of the labeled amount of metronidazole ($C_6H_9N_3O_3$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905)

- Procedure for content uniformity**
 - Diluent:** Diluted hydrochloric acid (1 in 100)
 - Standard solution:** 20 µg/mL of USP Metronidazole RS in *Diluent*
 - Sample stock solution:** Transfer 1 Tablet to a 250-mL volumetric flask. Add about 100 mL of *Diluent*, and shake for 30 min. Dilute with *Diluent* to volume. Filter, discarding the first 15 mL of the filtrate. Nominally 200 µg/mL of metronidazole is prepared by transferring the filtrate quantitatively with the *Diluent*.
 - Sample solution:** 20 µg/mL of metronidazole in *Diluent* from *Sample stock solution*
- Instrumental conditions**
 - Mode:** UV
 - Analytical wavelength:** 278 nm
 - Cell:** 1 cm
 - Blank:** *Diluent*
- Analysis**
 - Samples:** *Standard solution*, *Sample solution*, and *Blank*
 - Calculate the quantity, in mg, of metronidazole ($C_6H_9N_3O_3$) in each Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times L$$

- A_U = absorbance of the *Sample solution*
- A_S = absorbance of the *Standard solution*
- C_S = concentration of USP Metronidazole RS in the *Standard solution* (µg/mL)
- C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)
- L = label claim (mg/Tablet)

Acceptance criteria: Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase: Methanol and water (20:80)

Standard solution: 0.5 µg/mL of USP Metronidazole RS and 2.5 µg/mL of USP Tinidazole Related Compound A RS in *Mobile phase*

Sample solution: Nominally 500 µg/mL of metronidazole prepared as follows. Transfer a suitable amount of powdered Tablets (NLT 20) to a suitable volumetric flask. Add *Mobile phase* equivalent to 80% of the flask size. Sonicate for 10 min. Dilute with *Mobile phase* to volume, and pass a portion of the solution through a suitable filter. Use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 319 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 30 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 4.0 between metronidazole and tinidazole related compound A

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tinidazole related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of tinidazole related compound A from the *Sample solution*

r_S = peak response of tinidazole related compound A from the *Standard solution*

C_S = concentration of USP Tinidazole Related Compound A RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response of metronidazole from the *Standard solution*

C_S = concentration of USP Metronidazole RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metronidazole in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.7	0.5
Metronidazole	1.0	—
Any individual unspecified degradation product	—	0.10
Total impurities	—	2.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Metronidazole RS

USP Tinidazole Related Compound A RS

2-Methyl-5-nitroimidazole.

$C_4H_5N_3O_2$ 127.10

Metronidazole Extended-Release Tablets

DEFINITION

Metronidazole Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$).

IDENTIFICATION

• A. ULTRAVIOLET ABSORPTION (197U)

Diluent: Methanol and sulfuric acid (350:1)

Standard stock solution: 15 mg/mL of USP Metronidazole RS in dilute hydrochloric acid (1 in 100).

Sonicate to dissolve and pass through a suitable filter.

Standard solution: 18.8 µg/mL of USP Metronidazole RS in *Diluent* from *Standard stock solution*

Sample stock solution: Nominally 15 mg/mL of metronidazole prepared as follows. Finely powder NLT 5 Tablets and transfer an amount equivalent to 300 mg of metronidazole into a 20-mL volumetric flask. Add about 15 mL of dilute hydrochloric acid (1 in 100) and shake mechanically for 30 min. Dilute with dilute hydrochloric acid (1 in 100) to volume and shake well. Pass through a suitable filter.

Sample solution: Nominally equivalent to 18.8 µg/mL of metronidazole in *Diluent* from *Sample stock solution*

Wavelength range: 200–400 nm

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer: 1.4 g/L of monobasic potassium phosphate in water

Mobile phase: Methanol and *Buffer* (30:70)

Standard solution: 0.1 mg/mL of USP Metronidazole RS in *Mobile phase*

Sample stock solution: Nominally 2.0 mg/mL of metronidazole from NLT 20 finely powdered Tablets in *Mobile phase*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 60% of the flask volume with *Mobile phase*, and shake by mechanical means for 30 min. Dilute with *Mobile phase* to volume. Allow the solution to stand until the insoluble material settles.

Sample solution: Nominally 0.1 mg/mL of metronidazole in *Mobile phase* from the *Sample stock solution* supernatant. Pass the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 315 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Temperatures

Column: 30°

Autosampler: 15°

Flow rate: 1 mL/min

Injection volume: 10 μL

Run time: 15 min

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of metronidazole ($C_6H_9N_3O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of metronidazole from the *Sample solution* r_S = peak response of metronidazole from the *Standard solution* C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL) C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS**• DISSOLUTION (711)**

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 2, 6, 10, and 16 h

Standard solution: 16.65 μg/mL of USP Metronidazole RS in *Medium*Sample solution: At the times specified, withdraw 10 mL of the solution under test and pass through a suitable filter of 0.45-μm pore size. Replace the aliquots withdrawn for analysis with equal volumes of fresh portions of *Medium* maintained at 37°. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.Blank: *Medium***Instrumental conditions**

Mode: UV

Analytical wavelength: 320 nm

Cell: 1 cm

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the concentration (C_i) of metronidazole ($C_6H_9N_3O_3$) in the sample withdrawn from the vessel at each time point (i).

$$\text{Result} = (A_U/A_S) \times C_S \times D$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL) D = dilution factor, if neededCalculate the percentage of the labeled amount (Q_i) of metronidazole ($C_6H_9N_3O_3$) dissolved at each time point (i).

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = [(C_3 \times V) + [(C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

$$\text{Result}_4 = [(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

 C_i = concentration of metronidazole in the portion of sample withdrawn at the specified time point (mg/mL) V = volume of the *Medium*, 900 mL L = label claim (mg/Tablet) V_3 = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)Tolerances: See *Table 1*.**Table 1**

Time Point (i)	Time (h)	Amount Dissolved (%)
1	2	20–35
2	6	45–60
3	10	60–75
4	16	NLT 75

The percentages (Q) of the labeled amount of metronidazole ($C_6H_9N_3O_3$) released at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES**• ORGANIC IMPURITIES**

Buffer: Dissolve 1.5 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 3.2, and dilute with water to 1000 mL.

Diluent: Acetonitrile and Buffer (45:55)

Mobile phase: See *Table 2*.**Table 2**

Time (min)	Buffer (%)	Acetonitrile (%)
0	95	5
5	95	5
25	50	50
30	95	5
35	95	5

System suitability solution: 0.5 mg/mL of USP Metronidazole RS and 2.5 μg/mL of USP Tinidazole Related Compound A RS in *Diluent*. Sonicate, if necessary, to dissolve.**Standard solution:** 0.75 μg/mL of USP Metronidazole RS in *Diluent***Sample solution:** Nominally 0.5 mg/mL of metronidazole from NLT 20 finely powdered Tablets in *Diluent*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add *Diluent* equivalent to 70% of the flask volume, sonicate for 15 min with intermittent shaking, and dilute with *Diluent* to volume. Allow the solution to stand until the insoluble material settles, and pass the supernatant through a suitable filter of 0.45-μm pore size.

Chromatographic system(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 315 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Autosampler temperature: 20°

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitabilitySamples: *System suitability solution* and *Standard solution***Suitability requirements**Resolution: NLT 2.0 between tinidazole related compound A and metronidazole, *System suitability solution*Relative standard deviation: NMT 5.0% for metronidazole, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of each individual degradation product from the *Sample solution* r_S = peak response of metronidazole from the *Standard solution* C_S = concentration of USP Metronidazole RS in the *Standard solution* (mg/mL) C_U = nominal concentration of metronidazole in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 3. Disregard any impurity peaks less than 0.05%.

Table 3

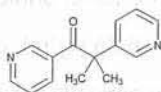
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tinidazole related compound A	0.79	0.15
Metronidazole	1.0	—
Any individual unspecified degradation product	—	0.10
Total degradation products	—	0.50

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.• **USP REFERENCE STANDARDS (11)**

USP Metronidazole RS

USP Tinidazole Related Compound A RS

2-Methyl-5-nitroimidazole.

C₄H₅N₃O₂ 127.10**Metrapone**C₁₄H₁₄N₂O

226.27

1-Propanone, 2-methyl-1,2-di-3-pyridinyl-;
2-Methyl-1,2-di-3-pyridyl-1-propanone [54-36-4].**DEFINITION**Metrapone contains NLT 98.0% and NMT 102.0% of metrapone (C₁₄H₁₄N₂O), calculated on the dried basis.**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197M)• **B. ULTRAVIOLET ABSORPTION** (197U)

Sample solution: 10 μg/mL in 1 N sulfuric acid

Acceptance criteria: Meets the requirements

ASSAY• **PROCEDURE**

Diluent: 1 N sulfuric acid

Standard solution: 10 μg/mL of USP Metrapone RS in Diluent

Sample solution: 10 μg/mL of Metrapone in Diluent

Instrumental conditions

Mode: UV

Analytical wavelength: 260 nm

Cell: 1 cm

Blank: Diluent

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of metrapone (C₁₄H₁₄N₂O) in the portion of Metrapone taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of USP Metrapone RS in the *Standard solution* (μg/mL) C_U = concentration of Metrapone in the *Sample solution* (μg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES• **RESIDUE ON IGNITION** (281): NMT 0.1%**Delete the following:**• **HEAVY METALS**, *Method II* (231): NMT 10 ppm • (Official 1-Jan-2018)• **ORGANIC IMPURITIES**

Standard stock solution: 0.2 mg/mL of USP Metrapone RS in methanol

Standard solution A: 40 μg/mL of USP Metrapone RS in methanol

Standard solution B: 20 μg/mL of USP Metrapone RS in methanol

Sample solution: 20 mg/mL of Metrapone in methanol

Chromatographic system(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μL

Developing solvent system: Chloroform and methanol (48:3)

AnalysisSamples: *Standard stock solution*, *Standard solution A*, *Standard solution B*, and *Sample solution*Apply each of the *Samples* separately to the TLC plate.Position the plate in a chromatographic chamber, and develop the chromatograms in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry under a stream of nitrogen for about 10 min. Position the dried plate once again in the same chromatographic chamber, and again develop the chromatograms until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry under a current of warm air for about 15 min. Examine the plate under short-wavelength UV light, and compare the

intensities of any secondary spots observed in the chromatogram of the *Sample solution* with those of the principal spots in the chromatograms of the *Standard solutions*.

Acceptance criteria: The intensity of any secondary spot from the *Sample solution* is NMT the principal spot from *Standard solution A* (0.2%), and the sum of the intensities of the secondary spots from the *Sample solution* corresponds to NMT 1.0%.

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry a sample under vacuum at room temperature for 6 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from heat and light.

• USP REFERENCE STANDARDS (11)

USP Metyrapone RS

Metyrapone Tablets

DEFINITION

Metyrapone Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of metyrapone ($C_{14}H_{14}N_2O$).

IDENTIFICATION

• A. INFRARED ABSORPTION

Sample solution: Transfer 500 mg of metyrapone from powdered Tablets into a centrifuge tube. Add 10 mL of 1 N sodium hydroxide, and mix. Extract with 10 mL of chloroform, centrifuge, and filter.

Acceptance criteria: The IR absorption spectrum of the *Sample solution*, determined in a 0.5-mm cell against chloroform, exhibits maxima only at the same wavelengths as that of a similar solution of USP Metyrapone RS.

• B. UV ABSORPTION

Sample solution: Transfer 1 mL of the filtrate obtained in *Identification test A* to a centrifuge tube. Add 20 mL of chloroform, and extract with 30 mL of 1 N sulfuric acid, centrifuging and filtering the sulfuric acid layer through a pledget of cotton. Mix 1 mL of this solution with 99 mL of 1 N sulfuric acid.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Metyrapone RS, concomitantly measured.

ASSAY

• PROCEDURE

Solution A: 13.3 mg/mL of 2,4-dinitrophenylhydrazine in methanol. Shake by mechanical means for about 15 min, and filter. Prepare fresh daily.

Solution B: *Solution A* and hydrochloric acid (23:2)

Solution C: 50 mg/mL of potassium hydroxide in methanol, and filter

Diluent: Chloroform and methanol (1:1)

Standard solution: 0.1 mg/mL of USP Metyrapone RS in chloroform

Sample stock solution: Nominally equivalent to 25 mg of metyrapone from powdered Tablets (NLT 20) prepared as follows. Transfer a suitable amount of powdered Tablets to a centrifuge tube with the aid of 10 mL of 1 N sodium hydroxide. Shake gently, and extract with three 15-mL portions of chloroform. Centrifuge each extract, filtering through a pledget of cotton, previously washed with chloroform, into a 50-mL volumetric flask. Add chloroform to volume, and mix.

Sample solution: Nominally 0.1 mg/mL from the *Sample stock solution* in chloroform

Instrumental conditions

Mode: Vis

Analytical wavelength: 450 nm

Cell: 1 cm

Analysis

Samples: *Standard solution*, *Sample solution*, and chloroform (blank)

Pipet 3 mL each of the *Standard solution*, the *Sample solution*, and the blank into separate 50-mL volumetric flasks. To each flask add 1 mL of *Solution B*, and shake lightly. Evaporate the solutions on a steam bath to near dryness. Wash down the sides of the flask with 1 mL of *Diluent*, and again evaporate the solutions to near dryness. Heat the flasks in an oven maintained at 110°–120° for 30 min. Remove the flasks, pipet 10 mL of *Solution C* into each flask, and heat again in the boiling water bath for 1 min. Allow to cool to room temperature, insert the stoppers, and shake by mechanical means for 5 min. Add methanol to volume, and mix. Measure the absorbance of the *Standard solution* and the *Sample solution* after the extraction against the blank.

Calculate the percentage of the labeled amount of metyrapone ($C_{14}H_{14}N_2O$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Metyrapone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metyrapone in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: Known concentration of USP Metyrapone RS in *Medium*

Sample solution: Portions of the solution under test suitably diluted with *Medium*, and filtered

Instrumental conditions

Mode: UV

Analytical wavelength: 259 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 60% (Q) of the labeled amount of metyrapone ($C_{14}H_{14}N_2O$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

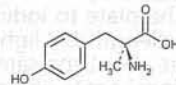
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

• USP REFERENCE STANDARDS (11)

USP Metyrapone RS

Metyrosine



$C_{10}H_{13}NO_3$ 195.22

L-Tyrosine, α -methyl-, (–)-.

(-)- α -Methyl-L-tyrosine [672-87-7].

» Metyrosine contains not less than 98.6 percent and not more than 101.0 percent of $C_{10}H_{13}NO_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Metyrosine RS

Identification—

A: Infrared Absorption (197M).

B: Ultraviolet Absorption (197U)—

Solution: 15 μ g per mL.

Medium: 0.1 N hydrochloric acid.

Absorptivities at 224 nm, calculated on the dried basis, do not differ by more than 3.0%.

Specific rotation (781S): between $+185^\circ$ and $+195^\circ$ ($t = 30^\circ$; $\lambda = 546$ nm; $l = 0.5$ dm).

Test solution: 5 mg per mL, in *Diluent*, with the aid of sonication if necessary. Prepare the *Diluent* as follows.

Solution A—Dissolve 20.0 g of anhydrous sodium acetate in about 150 mL of water in a 250-mL volumetric flask. Add 50.0 mL of glacial acetic acid, dilute with water to volume, and mix.

Solution B—Dissolve 62.5 g of cupric sulfate in water in a 200-mL volumetric flask, dilute with water to volume, and mix.

Diluent—Mix *Solution A* and *Solution B* in a 1000-mL volumetric flask, dilute with water to volume, and mix.

Loss on drying (731)—Dry it at a pressure not exceeding 5 mm of mercury at 100° for two hours: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals, Method II** (231): 0.003%. (Official 1-Jan-2018)

Chromatographic purity—

Standard solutions—Dissolve USP Metyrosine RS in a solvent mixture of methanol and ammonium hydroxide (7:3) to obtain a solution having a concentration of 10 mg per mL (*Standard solution A*). Pipet 1 mL of *Standard solution A* into a 100-mL volumetric flask, dilute with the same solvent mixture to volume, and mix (*Standard solution B*). Pipet 5 mL of *Standard solution B* into a 10-mL volumetric flask, dilute with the same solvent mixture to volume, and mix (*Standard solution C*). Pipet 5 mL of *Standard solution C* into a 10-mL volumetric flask, dilute with the same solvent mixture to volume, and mix (*Standard solution D*).

Test solution—Dissolve Metyrosine in the solvent mixture of methanol and ammonium hydroxide (7:3) to obtain a solution having a concentration of 10 mg per mL.

Procedure—Apply 10- μ L portions of *Standard solutions A, B, C, and D* and the *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture and previously washed with methanol. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-propyl alcohol and ammonium hydroxide (7:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate. Expose the plate to iodine vapors, and examine under short-wavelength UV light: the chromatogram shows principal spots at about the same R_f value. Estimate the levels of any additional spots observed in the chromatogram of the *Test solution* by comparison with the spots in the chromatograms of *Standard solutions B, C, and D*: the sum of the intensities of any spots observed is not greater

than that of the principal spot obtained from *Standard solution B*, corresponding to not more than 1%.

Assay—Dissolve about 300 mg of Metyrosine, accurately weighed, in about 100 mL of glacial acetic acid, sonicate for about 5 minutes, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a platinum ring electrode and a sleeve-type calomel electrode containing 0.1 N lithium perchlorate in glacial acetic acid (see *Titrimetry* (541)). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 19.52 mg of $C_{10}H_{13}NO_3$.

Metyrosine Capsules

» Metyrosine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of metyrosine ($C_{10}H_{13}NO_3$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Metyrosine RS

Identification—The UV absorption spectrum of a 1 in 10,000 solution of the Capsule contents in dilute hydrochloric acid (1 in 100) exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Metyrosine RS, concomitantly measured.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 750 mL.

Apparatus 1: 100 rpm.

Time: 60 minutes.

Procedure—Determine the amount of $C_{10}H_{13}NO_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 274 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Metyrosine RS in the same medium.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{10}H_{13}NO_3$ is dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Standard preparation—Dissolve a suitable quantity of USP Metyrosine RS, accurately weighed, in dilute hydrochloric acid (1 in 100) to obtain a solution having a known concentration of about 100 μ g per mL.

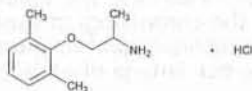
Assay preparation—Combine the contents of not less than 20 Capsules, and transfer an accurately weighed portion of the combined contents, equivalent to about 100 mg of metyrosine, to a 100-mL volumetric flask. Add 50 mL of dilute hydrochloric acid (1 in 100), shake by mechanical means for 45 minutes, dilute with dilute hydrochloric acid (1 in 100) to volume, mix, and filter. Transfer 10.0 mL of the filtrate to a 100-mL volumetric flask, dilute with dilute hydrochloric acid solution (1 in 100) to volume, and mix. Concomitantly determine the absorbances of this solution and the *Standard preparation* at the wavelength of maximum absorbance at about 274 nm, with a suitable spectrophotometer, using dilute hydrochloric acid solution (1 in 100) as the blank. Calculate the quantity, in mg, of metyrosine ($C_{10}H_{13}NO_3$) in the portion of Capsules taken by the formula:

$$C(A_U / A_S)$$

in which C is the concentration, in μ g per mL, of USP Metyrosine RS in the *Standard preparation*, and A_U and A_S

are the absorbances of the solutions obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Mexiletine Hydrochloride



$C_{11}H_{17}NO \cdot HCl$ 215.72
2-Propanamine, 1-(2,6-dimethylphenoxy)-, hydrochloride, (±)-;
(±)-1-Methyl-2-(2,6-xylyloxy)ethylamine hydrochloride [5370-01-04].

DEFINITION

Mexiletine Hydrochloride contains NLT 98.0% and NMT 102.0% of mexiletine hydrochloride ($C_{11}H_{17}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.**
Sample solution: 3 mL of a solution (1 in 60)
Analysis: Add 1 mL of 6 N ammonium hydroxide to the *Sample solution*, filter, and acidify the filtrate with 2 mL of nitric acid. Then add 1 mL of silver nitrate TS.
Acceptance criteria: A curdy, white precipitate is formed, and it is soluble in an excess of 6 N ammonium hydroxide (presence of chloride).

ASSAY

• PROCEDURE

Buffer: Dissolve 11.5 g of anhydrous sodium acetate in 500 mL of water. Add 3.2 mL of glacial acetic acid, mix, and allow to cool. Adjust with hydrochloric acid to a pH of 4.8 ± 0.1 , and dilute with water to 1000 mL.

Mobile phase: Methanol and *Buffer* (600:400)

Standard solution: 2 mg/mL of USP Mexiletine Hydrochloride RS in *Mobile phase*

System suitability solution: 1 mg/mL of 2-phenylethylamine hydrochloride in *Standard solution*

Sample solution: 2 mg/mL of Mexiletine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Columns

Guard: Packing L1

Analytical: 3.9-mm \times 30-cm; 10- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for 2-phenylethylamine and mexiletine are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the 2-phenylethylamine and mexiletine peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mexiletine hydrochloride ($C_{11}H_{17}NO \cdot HCl$) in the portion of Mexiletine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of mexiletine from the *Sample solution*

r_S = peak area of mexiletine from the *Standard solution*

C_S = concentration of USP Mexiletine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Mexiletine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 10 ppm (Official 1: Jan-2018)

• ORGANIC IMPURITIES

Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.2 mg/mL of USP Mexiletine Hydrochloride RS in *Mobile phase*, from the *Standard solution* in the *Assay*

Sample solution: 20 mg/mL of Mexiletine Hydrochloride in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 2-phenylethylamine and mexiletine are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the 2-phenylethylamine and mexiletine peaks, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Mexiletine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of mexiletine from the *Standard solution*

C_S = concentration of USP Mexiletine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Mexiletine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria

Any individual impurity: NMT 1%

Total impurities: NMT 1.5%

SPECIFIC TESTS• **pH (791)**

Sample solution: 100 mg/mL

Acceptance criteria: 3.5–5.5

• **LOSS ON DRYING (731)**

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in tight containers.• **USP REFERENCE STANDARDS (11)**

USP Mexiletine Hydrochloride RS

Mexiletine Hydrochloride Capsules

» Mexiletine Hydrochloride Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mexiletine hydrochloride ($C_{11}H_{17}NO \cdot HCl$).

Packaging and storage—Preserve in tight containers.**USP Reference standards (11)**—

USP Mexiletine Hydrochloride RS

Identification—

A: Transfer a quantity of Capsule contents, equivalent to about 250 mg of mexiletine hydrochloride, to a suitable test tube, add 10 mL of methanol, and mix on a vortex mixer for 1 minute. Filter the mixture, evaporate the filtrate under a stream of nitrogen to dryness, and dry the residue in vacuum at 60° for 1 hour: the IR absorption spectrum of a mineral oil dispersion of the dried residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Mexiletine Hydrochloride RS.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{11}H_{17}NO \cdot HCl$ dissolved from the difference between first derivative values at the wavelengths of maximum and minimum first derivative absorbance in the wavelength range from 230 to 290 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Mexiletine Hydrochloride RS in the same *Medium*.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{11}H_{17}NO \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Chromatographic purity—

Mobile phase, Standard preparation, and Resolution solution—Prepare as directed in the *Assay* under *Mexiletine Hydrochloride*.

Standard solution—Transfer 10.0 mL of the *Standard preparation* prepared as directed in the *Assay* under *Mexiletine Hydrochloride* to a 1000-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 20 µg of USP Mexiletine Hydrochloride RS per mL.

Test solution—Use the *Assay preparation* prepared as directed in the *Assay*.

Chromatographic system (see *Chromatography* (621))—Prepare as directed in the *Assay* under *Mexiletine Hydrochloride*, except that the relative standard deviation of replicate injections of the *Standard solution* is not more than 3.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph; record the chromatograms using a high sensitivity setting for the recorder; and measure the areas for the peaks. Calculate the percentage of each impurity observed by the formula:

$$100(C/L)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Mexiletine Hydrochloride RS in the *Standard solution*; L is the quantity, in mg, of mexiletine hydrochloride in each mL of the *Test solution*, based on the labeled amount in the portion of Capsule contents used to prepare the *Assay preparation* and the extent of dilution; r_U is the peak area obtained from an individual impurity observed in the chromatogram of the *Test solution*; and r_S is the mexiletine peak area obtained from the *Standard solution*: not more than 1% of any individual impurity is found; and the total of all observed impurities is not more than 1.5%.

Assay—

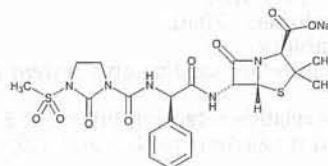
Mobile phase, Standard preparation, Resolution solution, and Chromatographic system—Prepare as directed in the *Assay* under *Mexiletine Hydrochloride*.

Assay preparation—Weigh the contents of not fewer than 20 Capsules, and calculate the average weight per Capsule. Mix the combined contents of the Capsules, and transfer an accurately weighed portion, equivalent to about 50 mg of mexiletine hydrochloride, to a stoppered, 50-mL centrifuge tube. Add 25.0 mL of *Mobile phase*, insert the stopper, and shake by mechanical means for 15 minutes. Centrifuge, and use the clear supernatant as the *Assay preparation*. [NOTE—Reserve a portion of this solution for use as the *Test solution* in the test for *Chromatographic purity*.]

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Mexiletine Hydrochloride*. Calculate the quantity, in mg, of mexiletine hydrochloride ($C_{11}H_{17}NO \cdot HCl$) in the portion of Capsule contents taken by the formula:

$$25C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Mexiletine Hydrochloride RS in the *Standard preparation*; and r_U and r_S are the mexiletine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Mezlocillin Sodium $C_{21}H_{24}NaN_5O_8S_2$

561.56

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-6-[[[3-(methylsulfonyl)-2-oxo-1-imidazolidinyl]carbonyl]amino]phenylacetyl] amino]-7-oxo-, monosodium salt, [2S-[2α,5α,6β (S*)]]-; Sodium (2S,5R,6R)-3,3-dimethyl-6-[(R)-2-[3-(methylsulfonyl)-2-oxo-1-imidazolidinecarboxamido]-2-phenylacetamido]-

7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate
[42057-22-7].
Monohydrate 579.58

DEFINITION

Mezlocillin Sodium contains the equivalent of NLT 838 µg/mg and NMT 978 µg/mg of mezlocillin ($C_{21}H_{25}N_5O_8S_2$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Sodium** (191): Meets the requirements

ASSAY

• PROCEDURE

Buffer: 4.9 g/L of monobasic potassium phosphate and 0.54 g/L of dibasic potassium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (145:855)

Standard solution: 0.5 mg/mL of mezlocillin from USP Mezlocillin Sodium RS in water

Sample solution: 0.55 mg/mL of Mezlocillin Sodium in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm × 12.5-cm; 5-µm packing L1

Temperature: 40°

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 1.5%

Tailing factor: NMT 1.5

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of mezlocillin ($C_{21}H_{25}N_5O_8S_2$) in each mg of Mezlocillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mezlocillin Sodium RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of mezlocillin in USP Mezlocillin Sodium RS (µg/mg)

Acceptance criteria: 838–978 µg/mg on the anhydrous basis

SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation** (781S)
Sample solution: 10 mg/mL in water
Acceptance criteria: +175° to +195°
- **pH** (791)
Sample solution: 100 mg/mL in water
Acceptance criteria: 4.5–8.0
- **WATER DETERMINATION, Method I** (921): NMT 6.0%
- **STERILITY TESTS** (71): Where the label states that Mezlocillin Sodium is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **BACTERIAL ENDOTOXINS TEST** (85): Where the label states that Mezlocillin Sodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.06 USP Endotoxin Units/mg of mezlocillin.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** (11)
USP Endotoxin RS
USP Mezlocillin Sodium RS

Mezlocillin for Injection

» Mezlocillin for Injection contains an amount of Mezlocillin Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of mezlocillin ($C_{21}H_{25}N_5O_8S_2$).

Change to read:

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1–May-2017).

USP Reference standards

USP Endotoxin RS

USP Mezlocillin Sodium RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

Bacterial Endotoxins Test (85)—It contains not more than 0.06 USP Endotoxin Unit per mg of mezlocillin.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to Be Examined*.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Other requirements—It responds to the *Identification tests* and meets the requirements for *Specific rotation, pH, and Water under Mezlocillin Sodium*. It meets also the requirements for *Uniformity of Dosage Units* (905) and *Labeling* (7), *Labels and Labeling for Injectable Products*.

Assay—

Mobile phase, Standard preparation, Resolution solution, and Chromatographic system—Prepare as directed for the *Assay under Mezlocillin Sodium*.

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Mezlocillin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution containing about 0.5 mg of mezlocillin per mL.

Assay preparation 2 (where the label states the quantity of mezlocillin in a given volume of constituted solution)—Constitute Mezlocillin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured portion of the constituted solution quantitatively with water to obtain a solution containing about 0.5 mg of mezlocillin per mL.

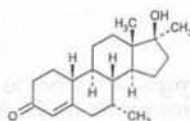
Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and *Assay preparation 1* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in

mg, of mezlocillin in the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C/1000)(r_U/r_S)$$

in which L is the labeled quantity, in mg, of mezlocillin in the container, or in the volume of constituted solution taken, D is the concentration, in mg per mL, of mezlocillin in Assay preparation 1 or in Assay preparation 2, on the basis of the labeled quantity in the container, or in the portion of constituted solution taken, respectively, and the extent of dilution, C is the concentration, in μg per mL, of mezlocillin ($\text{C}_{21}\text{H}_{25}\text{N}_5\text{O}_8\text{S}_2$) in the Standard preparation, and r_U and r_S are the mezlocillin peak responses obtained from the Standard preparation and from Assay preparation 1 or Assay preparation 2, as appropriate.

Mibolerone



$\text{C}_{20}\text{H}_{30}\text{O}_2$ 302.45
Estr-4-en-3-one, 17-hydroxy-7,17-dimethyl-, (7 α ,17 β)-
17 β -Hydroxy-7 α ,17-dimethylestr-4-en-3-one [3704-09-4].

» Mibolerone contains not less than 96.0 percent and not more than 106.0 percent of $\text{C}_{20}\text{H}_{30}\text{O}_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—
USP Mibolerone RS

Identification, Infrared Absorption (197M).

Specific rotation (781S): between +34° and +40°.

Test solution: 10 mg per mL, in chloroform.

Loss on drying (731)—Dry about 1 g, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.5%.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water, tetrahydrofuran, and methanol (60:25:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard solution—Prepare a solution of progesterone in methanol containing 0.6 mg per mL.

Standard preparation—Prepare a solution of USP Mibolerone RS in *Internal standard solution* having a known concentration of about 0.4 mg per mL. Mix, and sonicate if necessary to achieve complete solution.

Assay preparation—Transfer about 10 mg of Mibolerone, accurately weighed, to a 25-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix. Sonicate if necessary to achieve complete solution.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm \times 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about

0.6 for mibolerone and 1.0 for progesterone; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 5 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $\text{C}_{20}\text{H}_{30}\text{O}_2$ in the portion of Mibolerone taken by the formula:

$$25C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Mibolerone RS in the *Standard preparation*; and R_U and R_S are the ratios of the peak responses of the mibolerone peak and the progesterone peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Mibolerone Oral Solution

» Mibolerone Oral Solution contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of mibolerone ($\text{C}_{20}\text{H}_{30}\text{O}_2$).

Packaging and storage—Preserve in tight containers, protected from light.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—
USP Mibolerone RS

Identification—The chromatogram of the *Assay preparation* exhibits a major peak for mibolerone, the retention time of which corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Specific gravity (841): between 1.030 and 1.045.

Assay—

Internal standard solution—Prepare a solution of 1,3,5-triphenylbenzene in chloroform containing about 0.25 mg per mL.

Standard preparation—Prepare a solution of USP Mibolerone RS in *Internal standard solution* having a known concentration of about 0.5 mg per mL.

Assay preparation—Transfer an accurately weighed portion of Oral Solution, equivalent to about 1000 μg of mibolerone, to a 125-mL separator containing 60 mL of water, and swirl to disperse. Add 30 mL of methylene chloride, shake gently for about 5 minutes, and allow the phases to separate. Drain the lower methylene chloride layer through a pledget of methylene chloride-washed cotton into a 50-mL conical flask. Evaporate to dryness under a current of air. Re-extract the aqueous layer remaining in the separator with an additional 30-mL portion of methylene chloride, draining the filtered methylene chloride extract into the 50-mL conical flask, and evaporating it to dryness. Add 2.0 mL of *Internal standard solution*, and swirl to dissolve.

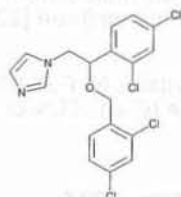
Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 3-mm \times 61-cm column packed with 1% liquid phase G6 on support S1AB. The column is maintained at about 175° and the detector at 195° to 225°. Helium is used as the carrier gas at a flow rate of about 60 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for the internal standard and 1.0 for mibolerone; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 2 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in μ g, of mibolerone ($C_{20}H_{30}O_2$) in each mL of the Oral Solution taken by the formula:

$$2000(C / W_U)(D)(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Mibolerone RS in the *Standard preparation*; *W_U* is the weight, in g, of Oral Solution taken to prepare the *Assay preparation*; *D* is the specific gravity of the Oral Solution; and *R_U* and *R_S* are the ratios of the peak height response of the mibolerone peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Miconazole



$C_{18}H_{14}Cl_4N_2O$

416.13

1*H*-Imidazole, 1-2-[(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]-, (±)-;
(±)-1-[2,4-Dichloro-β-[(2,4-dichlorobenzyl)oxy]phenethyl]imidazole [22916-47-8].

DEFINITION

Miconazole contains NLT 98.0% and NMT 102.0% of miconazole ($C_{18}H_{14}Cl_4N_2O$), calculated on the dried basis.

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

• B.

Sample solution: Dissolve 40 mg in 50 mL of isopropyl alcohol in a 100-mL volumetric flask, and add 10 mL of 0.1 N hydrochloric acid. Dilute with isopropyl alcohol to volume.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Miconazole RS, concomitantly measured.

ASSAY

• PROCEDURE

Sample solution: 300 mg of Miconazole in 40 mL of glacial acetic acid. Add 4 drops of *p*-naphtholbenzein TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with *Titrant* to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 41.61 mg of miconazole ($C_{18}H_{14}Cl_4N_2O$).

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• RESIDUE ON IGNITION (281): NMT 0.2%

• ORGANIC IMPURITIES

Standard solution A: 10 mg/mL of USP Miconazole RS in chloroform

Standard solution B: 100 μ g/mL of USP Miconazole RS from *Standard solution A* in chloroform

Sample solution: 10 mg/mL in chloroform

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Developing solvent system: *n*-Hexane, chloroform, methanol, and ammonium hydroxide (60:30:10:1)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* separately to the starting line of the plate. Develop the chromatogram in a suitable chamber with freshly prepared *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. Expose the plate to iodine vapors in a closed chamber for 30 min, and locate the spots.

Acceptance criteria: The *R_f* value of the principal spot from the *Sample solution* corresponds to that from *Standard solution A*, and any other spot from the *Sample solution* does not exceed, in size or intensity, the principal spot from *Standard solution B* (1.0%).

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry a sample under vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in well-closed containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.

• USP REFERENCE STANDARDS (11)

USP Miconazole RS

Miconazole Injection

DEFINITION

Miconazole Injection is a sterile solution of Miconazole in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of miconazole ($C_{18}H_{14}Cl_4N_2O$).

IDENTIFICATION

• A.

Solution A: 17 mg/mL of bismuth subnitrate in a mixture of glacial acetic acid and water (1:4)

Solution B: 400 mg/mL of potassium iodide in water

Standard solution: 5 mg/mL of USP Miconazole RS in methanol

Sample solution: Nominally 5 mg/mL of miconazole in methanol

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

Developing solvent system: *n*-Hexane, chloroform, methanol, and ammonium hydroxide (60:30:10:1)

Spray reagent (Dragendorff's TS): *Solution A*, *Solution B*, glacial acetic acid, and water (1:1:4:14)

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* separately to the starting line of the plate. Develop the chromatogram in a suitable chamber with freshly prepared *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. Locate the spots on the plate by spraying with *Spray reagent*.

Acceptance criteria: The R_f value of one of the principal spots from the *Sample solution* corresponds to that from the *Standard solution*.

ASSAY**• PROCEDURE**

Solution A: 25 mg/mL of ammonium acetate in water
Mobile phase: Acetonitrile, methanol, and *Solution A* (30:50:20)

System suitability solution: 50 µg/mL each of USP Miconazole RS and dibutyl phthalate in *Mobile phase*

Standard solution: 50 µg/mL of USP Miconazole RS in *Mobile phase*

Sample solution: Nominally 50 µg/mL of miconazole in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 30-cm; packing L7

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for dibutyl phthalate and miconazole are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5.0 between the dibutyl phthalate and miconazole peaks, *System suitability solution*

Tailing factor: NMT 1.3 for the miconazole peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the chromatograph to run for at least 16–18 min between injections to allow for elution of all components associated with the Injection vehicle.

Calculate the percentage of the labeled amount of miconazole ($C_{18}H_{14}Cl_4N_2O$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Miconazole RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of miconazole in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• pH (791): 3.7–5.7

• BACTERIAL ENDOTOXINS TEST (85): NMT 0.10 USP Endotoxin Unit/mg of miconazole

• PARTICULATE MATTER IN INJECTIONS (788): Meets the requirements for small-volume injections

• INJECTIONS AND IMPLANTED DRUG PRODUCTS (1): Meets the requirements

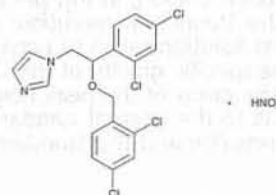
ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in single-dose containers, preferably of Type I glass, at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Endotoxin RS

USP Miconazole RS

Miconazole Nitrate

$C_{18}H_{14}Cl_4N_2O \cdot HNO_3$

479.14

1*H*-Imidazole, 1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]-, mononitrate; 1-[2,4-Dichloro-β-[(2,4-dichlorobenzyl)oxy]phenethyl]imidazole mononitrate [22832-87-7].

DEFINITION

Miconazole Nitrate contains NLT 98.0% and NMT 102.0% of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$), calculated on the dried basis.

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

• B. ULTRAVIOLET ABSORPTION (197U)

Sample solution: 400 µg/mL in a mixture of 0.1 N hydrochloric acid in isopropyl alcohol (1 in 10)

Acceptance criteria: Meets the requirements

ASSAY**• PROCEDURE**

Sample solution: 350 mg of Miconazole Nitrate in 50 mL of glacial acetic acid

Titrimetric system

Mode: Direct titration

Titant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Titrate the *Sample solution* with *Titant* using a glass-calomel electrode system. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 47.92 mg of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$).

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• RESIDUE ON IGNITION (281): NMT 0.2%

• ORGANIC IMPURITIES

Mobile phase: Methanol, acetonitrile, and 0.2 M ammonium acetate (32:30:38)

System suitability solution: 25 µg/mL each of USP Miconazole Nitrate RS and USP Econazole Nitrate RS in *Mobile phase*

Sample stock solution: 10 mg/mL of Miconazole Nitrate in *Mobile phase*

Sample solution: 25 µg/mL of Miconazole Nitrate, from *Sample stock solution*, in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

Run time: 1.2 times the retention time of the main peak

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for econazole and miconazole are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 10 between econazole and miconazole

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Sample stock solution* and *Sample solution*

Measure the responses of all peaks, excluding the peak representing nitrate ion and any peak producing a response less than 0.2 times the response of the main peak.

Acceptance criteria: The response of any individual peak, other than the main peak of the *Sample stock solution*, is NMT that of the main peak of the *Sample solution* (0.25%), and the sum of the responses of all peaks, other than the main peak of the *Sample stock solution*, is NMT twice the response of the main peak of the *Sample solution* (0.5%).

SPECIFIC TESTS

- Loss on Drying (731)**

Analysis: Dry a sample at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.
- USP REFERENCE STANDARDS (11)**
USP Econazole Nitrate RS
USP Miconazole Nitrate RS

Miconazole Nitrate Cream**DEFINITION**

Miconazole Nitrate Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$).

IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

- PROCEDURE**

Buffer: Triethylamine and water (10:1000). Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Methanol, acetonitrile, tetrahydrofuran, and *Buffer* (5:4:3:8)

Standard solution: 0.28 mg/mL of USP Miconazole Nitrate RS and 0.02 mg/mL of benzoic acid in *Mobile phase*

Sample solution: Nominally 0.28 mg/mL of miconazole nitrate in *Mobile phase* prepared as follows. Dissolve a weighed quantity of Cream in *Mobile phase*, and sonicate in a water bath at 40°–45° until the sample is completely dispersed. Cool the solution to below room temperature, and pass through a 0.45- μ m Teflon filter into an HPLC vial.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm \times 25-cm; packing L11

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 13 between miconazole nitrate and benzoic acid

Column efficiency: NLT 7500 theoretical plates for the miconazole nitrate peak

Tailing factor: NMT 2.0 for the miconazole nitrate peak

Relative standard deviation: NMT 2.0% from the miconazole nitrate peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Miconazole Nitrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of miconazole nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- MINIMUM FILL (755):** Meets the requirements

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, and store at controlled room temperature.
- LABELING:** Cream that is packaged and labeled for use as a vaginal preparation shall be labeled Miconazole Nitrate Vaginal Cream.
- USP REFERENCE STANDARDS (11)**
USP Miconazole Nitrate RS

Miconazole Nitrate Topical Powder**DEFINITION**

Miconazole Nitrate Topical Powder contains NLT 90.0% and NMT 110.0% of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$).

IDENTIFICATION

- A.**

Sample: Transfer nominally 100 mg of miconazole nitrate from Topical Powder to a 50-mL beaker, disperse in 40 mL of methanol, and mix for a minimum of 5 min. Allow to settle for 5–10 min, and filter into a 100-mL beaker. Evaporate on a steam bath to dryness. Dry the residue at 105° for 10 min.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue obtained from the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Miconazole Nitrate RS.

ASSAY• **PROCEDURE**

Internal standard solution: 0.5 mg/mL of cholestane in chloroform

Standard solution: 2 mg/mL of USP Miconazole Nitrate RS prepared as follows. Transfer 5.0 mL of 0.8 mg/mL of USP Miconazole Nitrate RS in a mixture of chloroform and methanol (1:1) to a test tube, and add 2.0 mL of *Internal standard solution*. Evaporate to dryness at a temperature not higher than 40° with the aid of a current of nitrogen. Dissolve the residue in 2.0 mL of a mixture of chloroform and methanol (1:1).

Sample solution: Nominally 2 mg/mL of miconazole nitrate prepared as follows. Transfer an equivalent to 20 mg of miconazole nitrate from Topical Powder to a 50-mL centrifuge tube. Add 25.0 mL of methanol, and shake by mechanical means for 30 min to dissolve the miconazole nitrate. Centrifuge to obtain a clear supernatant. Transfer 5.0 mL of this solution to a test tube, add 2.0 mL of *Internal standard solution*, and evaporate to dryness at a temperature not higher than 40° with the aid of a current of nitrogen. Dissolve the residue in 2.0 mL of a mixture of chloroform and methanol (1:1).

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.2-m glass; packed with 3% phase G32 on support S1A

Temperatures

Column: 250°

Injection port: 250°

Detector: 300°

Carrier gas: Helium

Flow rate: 50 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for cholestane and miconazole nitrate are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the cholestane and miconazole nitrate peaks

Relative standard deviation: NMT 3.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$) in the portion of Topical Powder taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of miconazole nitrate to cholestane from the *Sample solution*

R_S = peak response ratio of miconazole nitrate to cholestane from the *Standard solution*

C_S = concentration of USP Miconazole Nitrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of miconazole nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **MINIMUM FILL** (755): Meets the requirements

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total count does not exceed 10^2 cfu/g. It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)
USP Miconazole Nitrate RS

Miconazole Nitrate Vaginal Suppositories**DEFINITION**

Miconazole Nitrate Vaginal Suppositories contain NLT 90.0% and NMT 110.0% of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$).

IDENTIFICATION• **A.**

Sample: Place a portion of the *Sample stock solution* prepared as directed in the *Assay*, containing about 25 mg of miconazole nitrate, in a 50-mL beaker. Evaporate on a steam bath to dryness with the aid of a current of filtered air. Dry the residue at 105° for 10 min.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue obtained from the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Miconazole Nitrate RS.

ASSAY• **PROCEDURE**

Internal standard solution: 1 mg/mL of cholestane in a mixture of chloroform and methanol (1:1)

Standard solution: 2.5 mg/mL of USP Miconazole Nitrate RS prepared as follows. Transfer a 10.0-mL aliquot of a solution containing 500 µg/mL of USP Miconazole Nitrate RS in methanol to a test tube, and evaporate on a steam bath to dryness with the aid of a current of filtered air. Dissolve the residue in 2.0 mL of *Internal standard solution*.

Sample stock solution: Nominally 2.5 mg/mL of miconazole nitrate prepared as follows. Transfer 1 Suppository to a stoppered, 50-mL centrifuge tube. Add 30 mL of pentane, and shake by mechanical means for 20 min to dissolve the suppository base and to disperse the miconazole nitrate. Centrifuge to obtain a clear supernatant. Aspirate, and discard the clear liquid. Wash the residue with three 20-mL portions of pentane, shaking, centrifuging, and aspirating in the same manner. Discard the pentane washings. Evaporate the residual pentane from the residue with the aid of a current of filtered air. Using small portions of methanol, transfer the residue to a 100-mL volumetric flask. Dissolve in and dilute with methanol to volume.

Sample solution: Transfer an aliquot containing nominally the equivalent to 5 mg of miconazole nitrate from the *Sample stock solution* to a suitable container, and evaporate on a steam bath to dryness with the aid of a current of filtered air. Dissolve the residue in 2.0 mL of *Internal standard solution*.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.2-m glass; packed with 3% phase G32 on support S1A

Temperatures

Column: 250°

Injection port: 250°

Detector: 300°

Carrier gas: Helium

Flow rate: 50 mL/min

Injection volume: 1 µL

System suitabilitySample: *Standard solution*

[NOTE—The relative retention times for cholestane and miconazole nitrate are 0.44 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the cholestane and miconazole nitrate peaks

Relative standard deviation: NMT 3.0% for replicate injections

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$) in the portion of Suppository taken:

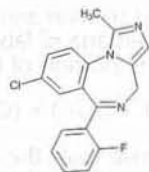
$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

 R_U = peak response ratio of miconazole nitrate to cholestane from the *Sample solution* R_S = peak response ratio of miconazole nitrate to cholestane from the *Standard solution* C_S = concentration of USP Miconazole Nitrate RS in the *Standard solution* (mg/mL) C_U = nominal concentration of miconazole nitrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Miconazole Nitrate RS

Midazolam

$C_{18}H_{13}ClFN_3$ 325.77
 4-*H*-Imidazo[1,5-*a*][1,4]benzodiazepine, 8-chloro-6-(2-fluorophenyl)-1-methyl;
 8-Chloro-6-(*o*-fluorophenyl)-1-methyl-4-*H*-imidazo[1,5-*a*][1,4]benzodiazepine [59467-70-8].

DEFINITIONMidazolam contains NLT 98.5% and NMT 101.5% of $C_{18}H_{13}ClFN_3$, calculated on the dried basis.**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY**• PROCEDURE**Buffer: 7.7 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 5.5 ± 0.1 .

Mobile phase: Acetonitrile and Buffer (1:2)

Standard solution: 0.04 mg/mL of USP Midazolam RS in *Mobile phase*Sample solution: 0.04 mg/mL of Midazolam in *Mobile phase***Chromatographic system**

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; 5-µm packing L60

Flow rate: 1.5 mL/min

Injection size: 25 µL

System suitabilitySample: *Standard solution***Suitability requirements**

Column efficiency: NLT 10,000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of $C_{18}H_{13}ClFN_3$ in the portion of Midazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Midazolam RS in the *Standard solution* (mg/mL) C_U = concentration of Midazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES**Inorganic Impurities**

- **RESIDUE ON IGNITION (281):** NMT 0.1%

Organic Impurities**• PROCEDURE**

Buffer, Mobile phase, Standard solution, and Chromatographic system: Proceed as directed in the Assay.

Sensitivity check solution: Dilute the *Standard solution* with *Mobile phase* to obtain a 0.2-µg/mL solution.Sample solution: 0.2 mg/mL of Midazolam in *Mobile phase***System suitability**Samples: *Standard solution* and *Sensitivity check solution***Suitability requirements**Column efficiency: NLT 10,000 theoretical plates, *Standard solution*Tailing factor: NMT 2.0, *Standard solution*Relative standard deviation: NMT 2.0%, *Standard solution*Peak ratio: The ratio of the area of the midazolam peak of the *Standard solution* to the area of the midazolam peak of the *Sensitivity check solution* should be within 160–240.**Analysis**Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Midazolam taken:

$$\text{Result} = (r_U/F)/[\Sigma(r_U/F) + r_T] \times 100$$

 r_U = peak response of each individual impurity from the *Sample solution* r_T = peak response of Midazolam from the *Sample solution* F = relative response factor (see Impurity Table 1)

Acceptance criteria: See Impurity Table 1.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Reduced midazolam ^a	0.20	1.0	0.1
Reduced reduced midazolam ^b	0.24	1.0	0.1
Amino compound ^c	0.25	0.5	0.1
Oxide midazolam ^d	0.46	1.3	0.1
Nitromethylene compound ^e	0.76	1.0	0.1
Dihydromidazolam ^f	0.83	0.5	0.1
Midazolam	1.0	—	—
Desfluoromidazolam ^g	1.14	1.0	0.2
6H-isomer ^h	2.48	0.7	0.1
Unknown impurity	—	1.0	0.1
Total impurities	—	—	0.5

^a 8-Chloro-3a,4-dihydro-6-(2-fluorophenyl)-1-methyl-3H-imidazo[1,5-a][1,4]-benzodiazepine.

^b 8-Chloro-6-(2-fluorophenyl)-3a,4,5,6-tetrahydro-1-methyl-3H-imidazo[1,5-a][1,4]-benzodiazepine.

^c 2-Aminomethyl-7-chloro-2,3-dihydro-5-(2-fluorophenyl)-1H-1,4-benzodiazepine.

^d 8-Chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo[1,5-a][1,4]-benzodiazepine-5-oxide.

^e 7-Chloro-1,3-dihydro-2-nitromethylene-5-(2-fluorophenyl)-2H-1,4-benzodiazepine-4-oxide.

^f 8-Chloro-6-(2-fluorophenyl)-5,6-dihydro-1-methyl-4H-imidazo[1,5-a][1,4]-benzodiazepine.

^g 8-Chloro-6-phenyl-1-methyl-4H-imidazo-[1,5-a][1,4]-benzodiazepine.

^h 8-Chloro-6-(2-fluorophenyl)-1-methyl-6H-imidazo[1,5-a][1,4]-benzodiazepine.

SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
USP Midazolam RS

Midazolam Injection

DEFINITION

Midazolam Injection is a sterile solution of Midazolam Hydrochloride in Water for Injection or of Midazolam in Water for Injection prepared with the aid of Hydrochloric Acid. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of midazolam ($C_{18}H_{13}ClFN_3$). It may contain Sodium Chloride, Benzyl Alcohol, and/or a chelating agent.

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

[NOTE—Protect all prepared Standard and sample solutions from light.]

PROCEDURE

Buffer: 6.7 g/L of dibasic sodium phosphate heptahydrate in water. Adjust with phosphoric acid to a pH of 5.0 ± 0.1 .

Solution A: Prepare a filtered and degassed mixture of acetonitrile, methanol and *Buffer* (8:3:9).

Solution B: Acetonitrile and *Buffer* (3:1)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
20	0	100
35	0	100
37	100	0
45	100	0

Standard solution: Dissolve USP Midazolam RS in about 2 mL of methanol, and dilute quantitatively, and stepwise if necessary, with *Solution A* to obtain a 0.2-mg/mL solution.

Sample solution: [NOTE—The midazolam present in the Injection converts from the open-ring form to the closed-ring form when diluted with *Solution A*. The midazolam potency is determined based on the peak area of the closed-ring form. It takes approximately 60 min at 40° or 2–3 h at room temperature to complete the conversion. The *Standard solution* is not subject to this conversion process.] Transfer a volume of Injection to a suitable volumetric flask, and dilute with *Solution A* to obtain a solution containing about 0.2 mg/mL of midazolam. Transfer the resulting solution into suitable crimp top vials, seal tightly, and heat at about 40° for 60 min. Allow this solution to cool to room temperature before injection.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; packing L1

Flow rate: 1.0 mL/min

Injection size: 50 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 5500 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of labeled amount of $C_{18}H_{13}ClFN_3$ in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Midazolam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of Midazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Organic Impurities

[NOTE—Protect all prepared Standard and sample solutions from light.]

PROCEDURE

Buffer, Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard stock solution: Use *Standard solution* in the *Assay*.

Standard solution: 0.5 μ g/mL USP Midazolam RS in *Solution A* from *Standard stock solution*

Control solution: 0.1 μ g/mL USP Midazolam RS in *Solution A* from *Standard solution*

System suitability

Samples: *Standard solution* and *Control solution*

Suitability requirements

Tailing factor: NMT 2.5 for midazolam peak, *Standard solution*

Column efficiency: NLT 5500 theoretical plates, *Standard solution*

Signal-to-noise ratio: NLT 10, *Control solution*

Relative standard deviation: NMT 8.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of the individual impurity from the *Sample solution*

r_s = peak response of midazolam from the *Standard solution*

C_s = concentration of USP Midazolam RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of Midazolam in the *Sample solution* (mg/mL)

F = relative response factor; 0.51 for the peak eluting at a relative retention between 0.79 and 0.97 with respect to midazolam; 1.0 for all other peaks

Acceptance criteria

Individual known impurity: NMT 0.5%

Individual unknown impurity: NMT 0.1%

Total impurities: NMT 1.0%

[NOTE—Disregard all solvent- and excipient-related peaks.]

SPECIFIC TESTS**• BENZYL ALCOHOL CONTENT** (if present)

Buffer: 3.4 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Acetonitrile and *Buffer* (7:13)

System suitability solution: 0.05 mg/mL of USP Midazolam RS and 0.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*

Standard solution: 0.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*

Sample solution: Transfer a measured volume of Injection to a suitable volumetric flask. Dilute with *Mobile phase* to obtain a concentration of about 0.5 mg/mL of benzyl alcohol, based on the labeled content of benzyl alcohol in the Injection.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; L1 packing

Flow rate: 1.0 mL/min

Injection size: 50 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 6.0 between benzyl alcohol and midazolam

Tailing factor: NMT 2.0 for benzyl alcohol

Relative standard deviation: NMT 2.0% for benzyl alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzyl alcohol in the volume of Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of benzyl alcohol from the *Sample solution*

r_s = peak response of benzyl alcohol from the *Standard solution*

C_s = concentration of USP Benzyl Alcohol RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of benzyl alcohol in the *Sample solution* (mg/mL)

Acceptance criteria: The content of benzyl alcohol meets the requirements in *Injections and Implanted Drug Products* (1), *Specific Tests*, *Vehicles and added substances*.

• **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections

• **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 8.33 USP Endotoxin Units/mg of midazolam.

• **PH** (791): 2.5–3.7

• **STERILITY TESTS** (71): Meets the requirements

• **OTHER REQUIREMENTS:** It meets the requirements for *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type 1 glass. Store between 15° and 30°.

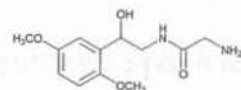
• **LABELING:** Label to indicate the vehicle used and the names and concentrations of any added preservatives. Indicate if the product is preservative free.

• **USP REFERENCE STANDARDS** (11)

USP Benzyl Alcohol RS

USP Endotoxin RS

USP Midazolam RS

Midodrine Hydrochloride

$C_{12}H_{18}N_2O_4 \cdot HCl$ 290.74

Acetamide, 2-amino-N-[2-(2,5-dimethoxyphenyl)-2-hydroxyethyl]-, monohydrochloride, (±)-;

(±)-2-Amino-N-(β-hydroxy-2,5-dimethoxyphenethyl)acetamide monohydrochloride [3092-17-9].

DEFINITION

Midodrine Hydrochloride contains NLT 98.0% and NMT 102.0% of midodrine hydrochloride ($C_{12}H_{18}N_2O_4 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

• **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): A 10 mg/mL solution of Midodrine Hydrochloride in water meets the requirements.

ASSAY**• PROCEDURE**

Buffer: 13.6 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 4.00 ± 0.05 .

Mobile phase: Acetonitrile and *Buffer* (3:22)

Standard solution: 0.05 mg/mL of USP Midodrine Hydrochloride RS in *Mobile phase*

Sample solution: 0.05 mg/mL of Midodrine Hydrochloride in *Mobile phase*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection size: 20 μL

System suitabilitySample: *Standard solution***Suitability requirements**

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of $C_{12}H_{18}N_2O_4 \cdot HCl$ in the portion of Midodrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of midodrine from the *Sample solution* r_S = peak response of midodrine from the *Standard solution* C_S = concentration of USP Midodrine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = concentration of Midodrine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES**Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.2%. A 1-g sample is used.

Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 10 ppm (Official 1-Jan-2018)

Organic Impurities**• PROCEDURE**Buffer and Mobile phase: Proceed as directed in the *Assay*.Standard solution: 1.0 μg/mL of USP Midodrine Hydrochloride RS and 2.0 μg/mL of USP Midodrine Related Compound A RS in *Mobile phase*Sample solution: 1.0 mg/mL of Midodrine Hydrochloride in *Mobile phase*Chromatographic system: Proceed as directed in the *Assay* except for the following:

Injection size: 50 μL

System suitabilitySample: *Standard solution***Suitability requirements**

Resolution: NLT 2.0 between midodrine hydrochloride and midodrine hydrochloride related compound A

Tailing factor: NMT 2.0 for midodrine hydrochloride

Relative standard deviation: NMT 2.0% for both midodrine hydrochloride and midodrine related compound A

AnalysisSamples: *Standard solution* and *Sample solution*

Calculate the percentage of midodrine related compound A in the portion of Midodrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of midodrine related compound A from the *Sample solution* r_S = peak response of midodrine related compound A from the *Standard solution* C_S = concentration of USP Midodrine Related Compound A RS in the *Standard solution* (mg/mL) C_U = concentration of Midodrine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual impurity in the portion of Midodrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_S = peak response of midodrine from the *Standard solution* C_S = concentration of USP Midodrine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = concentration of Midodrine Hydrochloride in the *Sample solution* (mg/mL)Acceptance criteria: See *Impurity Table 1*.**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Midodrine related compound A ^a	0.8	0.2%
Midodrine hydrochloride	1	—
Individual unspecified impurity	—	0.1%
Total impurities	—	0.5%

^a 1-(2,5 Dimethoxyphenyl)-2-aminoethanol.**SPECIFIC TESTS**

- **WATER DETERMINATION**, *Method I* (921): NMT 0.5%
- **PH** (791): 4.0–5.0. Use 50 mg/mL of the midodrine hydrochloride sample.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers and store at room temperature.
- **USP REFERENCE STANDARDS** (11)
 - USP Midodrine Hydrochloride RS
 - USP Midodrine Related Compound A RS
 - 1-(2,5 Dimethoxyphenyl)-2-aminoethanol.
 - $C_{10}H_{15}NO_3$ 197.23

Midodrine Hydrochloride Tablets**DEFINITION**Midodrine Hydrochloride Tablets contain NLT 90.0% and NMT 105.0% of the labeled amount of Midodrine Hydrochloride ($C_{12}H_{18}N_2O_4 \cdot HCl$).**IDENTIFICATION****• A. INFRARED ABSORPTION (197K)**

Sample specimen: Weigh a quantity, from finely powdered Tablets (NLT 20), equivalent to 15 mg of midodrine hydrochloride, into a 50-mL disposable centrifuge tube. Add 20 mL of water, and stir for 2 min using a vortex mixer. Pass the mixture through filter paper into a 50-mL beaker, and boil it until about 2 mL of the solution is left. Evaporate the final solution in an oven at 105° for 1 h.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Buffer: 13.6 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 4.00 ± 0.05 .

Mobile phase: Acetonitrile and Buffer (3:22)

Standard solution: 0.05 mg/mL of USP Midodrine Hydrochloride RS in *Mobile phase*

Sample solution: 0.05 mg/mL of midodrine hydrochloride in *Mobile phase* from NLT 5 Tablets (for 10-mg Tablet strength) or NLT 10 Tablets (for 5-mg and 2.5-mg Tablet strength). Initially add *Mobile phase* up to 80% of the volume of the flask. Sonicate for 10 min, stir for 15 min, and then dilute to volume, mix, and let stand for 10 min. Pass through a suitable PVDF filter of 0.45- μ m pore size, and discard the first 5 mL.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 290 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1.0 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of midodrine hydrochloride ($C_{12}H_{18}N_2O_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–105.0%

PERFORMANCE TESTS**• DISSOLUTION (711)**

Medium: 0.1 N HCl; 900 mL, deaerated

Apparatus 2: 50 rpm

Time: 15 min

Buffer: Proceed as directed in the Assay.

Mobile phase: Acetonitrile and Buffer (3:17)

Standard solution: L/900 mg/mL of USP Midodrine Hydrochloride RS in *Medium*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 45- μ m pore size.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 290 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1.0 mL/min

Injection size: 50 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of midodrine hydrochloride dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of midodrine hydrochloride is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

IMPURITIES**Organic Impurities****• PROCEDURE**

Buffer and Mobile phase: Proceed as directed in the Assay.

Standard stock solution 1: 25 μ g/mL of USP

Midodrine Hydrochloride RS in *Mobile phase*

Standard stock solution 2: 25 μ g/mL of USP

Midodrine Related Compound A RS in *Mobile phase*

Standard solution: 1.25 μ g/mL each of USP Midodrine Hydrochloride RS and USP Midodrine Related Compound A RS in *Mobile phase* from *Standard stock solution 1* and *Standard stock solution 2*

Sample solution: 0.25 mg/mL in *Mobile phase* from NLT 5 Tablets (for 10-mg Tablet strength) and NLT 10 Tablets (for 5-mg and 2.5-mg Tablet strength). Initially add *Mobile phase* to about 80% of the volume of the flask. Sonicate for 10 min, stir for 15 min, and then dilute to volume. Pass through a suitable PVDF filter of 0.45- μ m pore size, and discard the first 5 mL.

Chromatographic system

(See Chromatography (621), System Suitability.)

Proceed as directed in the Assay except for the following:

Injection volume: 40 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

[NOTE—The relative retention times for midodrine related compound A and midodrine hydrochloride are 0.83 and 1, respectively.]

Resolution: NLT 2.0 between midodrine hydrochloride and midodrine related compound A

Column efficiency: NLT 2000 theoretical plates for the midodrine peak

Tailing factor: NMT 2.0 for the midodrine peak

Relative standard deviation: NMT 2.0% for the midodrine peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of midodrine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of midodrine related compound A from the *Sample solution*

r_S = peak response of midodrine related compound A from the *Standard solution*

C_S = concentration of USP Midodrine Related Compound A RS in the *Standard solution* (μ g/mL)

C_U = nominal concentration of midodrine hydrochloride in the *Sample solution* (μ g/mL)

Calculate the percentage of any other unknown impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other unknown impurity from the *Sample solution*

r_S = peak response of midodrine from the *Standard solution*

- C_s = concentration of USP Midodrine Hydrochloride RS in the *Standard solution* ($\mu\text{g/mL}$)
 C_u = nominal concentration of midodrine hydrochloride in the *Sample solution* ($\mu\text{g/mL}$)

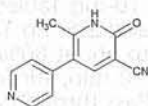
Acceptance criteria

Individual impurities: NMT 0.5% of midodrine related compound A; NMT 0.2% of any other individual impurity

Total impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
 USP Midodrine Hydrochloride RS
 USP Midodrine Related Compound A RS
 1-(2,5-Dimethoxyphenyl)-2-aminoethanol.
 $\text{C}_{10}\text{H}_{15}\text{NO}_3$ 197.23

Milrinone

$\text{C}_{12}\text{H}_9\text{N}_3\text{O}$ 211.22
 [3,4'-Bipyridine]-5-carbonitrile, 1,6-dihydro-2-methyl-6-oxo-;
 1,6-Dihydro-2-methyl-6-oxo[3,4'-bipyridine]-5-carbonitrile
 [78415-72-2].

DEFINITION

Milrinone contains NLT 98.0% and NMT 102.0% of milrinone ($\text{C}_{12}\text{H}_9\text{N}_3\text{O}$), calculated on the anhydrous basis.

[CAUTION—Milrinone is a cardiotonic agent.]

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Buffer: To 72.44 g of sodium tetraborate add 900 mL of water. Adjust with hydrochloric acid to a pH of 6.5. The solution should become nearly transparent after adjustment. Dilute with water to 1 L.

Mobile phase: Methanol, *Buffer*, and water (320:40:640)

Diluent: Methanol, water, and lactic acid (320:679:1.2)

Standard solution: 0.1 mg/mL of USP Milrinone RS in *Diluent*. Sonicate until dissolved.

Sample solution: 0.1 mg/mL of Milrinone in *Diluent*. Sonicate until dissolved.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 268 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of milrinone ($\text{C}_{12}\text{H}_9\text{N}_3\text{O}$) in the portion of Milrinone taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of milrinone from the *Sample solution*

r_s = peak response of milrinone from the *Standard solution*

C_s = concentration of USP Milrinone RS in the *Standard solution* (mg/mL)

C_u = concentration of Milrinone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.2%

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-Jan-2018)

• ORGANIC IMPURITIES

Buffer: To 2.7 g of dibasic potassium phosphate in 800 mL of water add 2.4 mL of triethylamine, and adjust with phosphoric acid to a pH of 7.5.

Mobile phase: Acetonitrile and *Buffer* (200:800)

System suitability stock solution: 0.2 mg/mL of USP Milrinone Related Compound A RS in *Mobile phase*.

Heat in a water bath at approximately 80°, and/or sonicate if necessary to dissolve.

Standard stock solution: 2 mg/mL of USP Milrinone RS in *Mobile phase*. Heat in a water bath at approximately 80°, and/or sonicate if necessary to dissolve.

System suitability solution: 10.0 mL of *System suitability stock solution* and 1.0 mL of *Standard stock solution* in a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.

Standard solution: 0.006 mg/mL of USP Milrinone RS, from the *Standard stock solution*, in *Mobile phase*

Sample solution: 2 mg/mL of Milrinone in *Mobile phase*. Heat in a water bath at approximately 80°, if necessary to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 25-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for milrinone related compound A and milrinone are 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between milrinone related compound A and milrinone

Relative standard deviation: NMT 5.0% from the milrinone peak

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Milrinone taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- r_u = peak response of each impurity from the *Sample solution*
 r_s = peak response of milrinone from the *Standard solution*
 C_s = concentration of USP Milrinone RS in the *Standard solution* (mg/mL)
 C_u = concentration of Milrinone in the *Sample solution* (mg/mL)

Acceptance criteria

Any individual impurity: NMT 0.3%
 Total impurities: NMT 1.0%

SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
 - USP Milrinone RS
 - USP Milrinone Related Compound A RS
 - 1,6-Dihydro-2-methyl-6-oxo-(3,4'-bipyridine)-5-carboxamide.
 - $C_{12}H_{11}N_3O_2$ 229.23

Mineral Oil

DEFINITION

Mineral Oil is a purified mixture of liquid hydrocarbons obtained from petroleum. It may contain a suitable stabilizer.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197F)**
- **B.** It meets the requirements in *Specific Tests for Viscosity—Capillary Methods* (911).

IMPURITIES

- **LIMIT OF POLYCYCLIC AROMATIC HYDROCARBONS**

Dimethyl sulfoxide: Use spectrophotometric grade dimethyl sulfoxide.

n-Hexane: Use n-hexane that has been washed by being shaken previously twice with one-fifth its volume of *Dimethyl sulfoxide*.

Standard solution: 7.0 µg/mL of USP Naphthalene RS in isooctane (2,2,4-trimethylpentane)

Standard blank: 2,2,4-Trimethylpentane

Sample solution: Transfer 25.0 mL of Mineral Oil and 25 mL of n-Hexane to a 125-mL separator, and mix.
 [NOTE—Use no lubricants other than water on the stopcock, or use a separator equipped with a suitable polymeric stopcock.]

Add 5.0 mL of *Dimethyl sulfoxide*, and shake the mixture vigorously for 1 min. Allow to stand until the lower layer is clear, transfer the lower layer to another 125-mL separator, add 2 mL of n-Hexane, and shake vigorously. Use the lower layer.

Sample blank: *Dimethyl sulfoxide* that has been shaken previously vigorously for 1 min with n-Hexane in the ratio of 5 mL of *Dimethyl sulfoxide* to 25 mL of n-Hexane

Instrumental conditions

Mode: UV

Analytical wavelengths

Standard solution: 275 nm

Sample solution: 260–350 nm

Cell: 1 cm

Analysis

Samples: *Standard solution*, *Standard blank*, *Sample solution*, and *Sample blank*

Acceptance criteria: The absorbance at any wavelength in the specified range of the *Sample solution* is NMT one-third of the absorbance of the *Standard solution*.

SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 0.845–0.905
- **VISCOSITY—CAPILLARY METHODS (911):** 34.5–150.0 mm²·s⁻¹ for kinematic viscosity, measured with a capillary viscometer at 40 ± 0.1°
- **ACIDITY**

Sample: 10 mL

Analysis: Add 20 mL of boiling water to the *Sample*, and shake vigorously for about 1 min. Allow to cool, and draw off the separated water. To 10 mL of the filtered aqueous layer add 0.1 mL of phenolphthalein TS.

Acceptance criteria: The solution does not produce a pink color. NMT 1.0 mL of 0.01 N sodium hydroxide is required to produce a pink color.
- **READILY CARBONIZABLE SUBSTANCES TEST (271)**

Sample: 5 mL

Standard solution: In a glass-stoppered test tube that previously has been rinsed with hot nitric acid (see *Cleaning Glass Apparatus* (1051)), mix 3 mL of ferric chloride CS, 1.5 mL of cobaltous chloride CS, and 0.5 mL of cupric sulfate CS then overlaid with 5 mL of Mineral Oil.

Analysis: Place the *Sample* in a glass-stoppered test tube that previously has been rinsed with hot nitric acid (see *Cleaning Glass Apparatus* (1051)), then rinsed with water, and dried. Add 5 mL of sulfuric acid containing 94.5%–94.9% of H₂SO₄, and heat in a boiling water bath for 10 min. After the test tube has been in the bath for 30 s, remove it quickly, and while holding the stopper in place, give three vigorous, vertical shakes over an amplitude of about 5 in. Repeat every 30 s. Do not keep the test tube out of the bath longer than 3 s for each shaking period. At the end of 10 min from the time when first placed in the water bath, remove the test tube.

Acceptance criteria: The oil portion of the *Sample* may turn hazy, but it remains colorless or shows a slight pink or yellow color, and the acid portion of the *Sample* does not become darker than the *Standard solution*.
- **SOLID PARAFFIN**

Sample: Mineral Oil that has been dried previously in a beaker at 105° for 2 h and cooled to room temperature in a desiccator over silica gel

Analysis: Fill a tall, cylindrical, standard oil-sample bottle of colorless glass of 120-mL capacity with the *Sample*, insert the stopper, and immerse in an ice bath for 4 h.

Acceptance criteria: The *Sample* is sufficiently clear that a black line 0.5 mm in width, on a white background, held vertically behind the bottle, is clearly visible.
- **LIMIT OF SULFUR COMPOUNDS**

Solution A: Saturated solution of lead(II) oxide in sodium hydroxide (200 mg/mL)

Sample: 4.0 mL

Analysis: Combine the *Sample*, 2 mL of dehydrated alcohol, and 2 drops of *Solution A*, heat at 70° for 10 min with frequent shaking, and cool.

Acceptance criteria: No dark brown color develops.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. No storage requirements specified.
- **LABELING:** Label it to indicate the name and quantity of any substance added as a stabilizer.
- **USP REFERENCE STANDARDS (11)**
USP Mineral Oil RS
USP Naphthalene RS

Mineral Oil Emulsion

DEFINITION

Prepare Mineral Oil Emulsion as follows.

Mineral Oil	500 mL
Acacia, in very fine powder	125 g
Syrup	100 mL
Vanillin	40 mg
Alcohol	60 mL
Purified Water, a sufficient quantity to make	1000 mL

Mix the *Mineral Oil* with the *Acacia* in a dry mortar. Add 250 mL of *Purified Water* all at once, and emulsify the mixture. Add, in divided portions, triturating after each addition, a mixture of the *Syrup*, 50 mL of *Purified Water*, and *Vanillin* dissolved in *Alcohol*. Add *Purified Water* to bring the preparation to final volume, and mix.

The *Vanillin* may be replaced by NMT 1% of any other official flavoring substance or any mixture of official flavoring substances. *Alcohol* may be replaced with 60 mL of sweet orange peel tincture or 2 g of benzoic acid as a preservative.

SPECIFIC TESTS

- **ALCOHOL DETERMINATION, Method I (611):** 4.0%–6.0% of C_2H_5OH

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight containers.

Mineral Oil, Rectal

DEFINITION

Mineral Oil, Rectal, is Mineral Oil that has been suitably packaged.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197F)**
- **B.** It meets the requirements in *Specific Tests for Viscosity—Capillary Methods (911)*.

SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 0.845–0.905
- **VISCOSITY—CAPILLARY METHODS (911):** 34.5–150.0 $mm^2 \cdot s^{-1}$ for kinematic viscosity, measured with a capillary viscometer at $40 \pm 0.1^\circ$
- **ACIDITY**
Sample: 10 mL
Analysis: Add 20 mL of boiling water to the *Sample*, and shake vigorously for about 1 min. Allow to cool, and draw off the separated water. To 10 mL of the filtered aqueous layer add 0.1 mL of phenolphthalein TS.

Acceptance criteria: The solution does not produce a pink color. NMT 1.0 mL of 0.01 N sodium hydroxide is required to produce a pink color.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant, single-unit containers. No storage requirements specified.
- **USP REFERENCE STANDARDS (11)**
USP Mineral Oil RS

Topical Light Mineral Oil

DEFINITION

Topical Light Mineral Oil is Light Mineral Oil that has been suitably packaged.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197F)**
- **B.** It meets the requirements in *Specific Tests for Viscosity—Capillary Methods (911)*.

SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 0.818–0.880
- **VISCOSITY—CAPILLARY METHODS (911):** 3.0–34.4 $mm^2 \cdot s^{-1}$ for kinematic viscosity, measured with a capillary viscometer at $40 \pm 0.1^\circ$
- **ACIDITY**
Sample: 10 mL
Analysis: Add 20 mL of boiling water to the *Sample*, and shake vigorously for about 1 min. Allow to cool, and draw off the separated water. To 10 mL of the filtered aqueous layer add 0.1 mL of phenolphthalein TS.
Acceptance criteria: The solution does not produce a pink color. NMT 1.0 mL of 0.01 N sodium hydroxide is required to produce a pink color.
- **SOLID PARAFFIN**
Sample: Topical Light Mineral Oil that has been dried previously in a beaker at 105° for 2 h and cooled to room temperature in a desiccator over silica gel
Analysis: Fill a tall, cylindrical, standard oil-sample bottle of colorless glass of 120-mL capacity with the *Sample*. Insert the stopper, and immerse the bottle in a mixture of ice and water for 4 h.
Acceptance criteria: The *Sample solution* is sufficiently clear that a black line 0.5 mm in width, on a white background, held vertically behind the bottle, is clearly visible.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. No storage requirements specified.
- **LABELING:** Label it to indicate the name and quantity of any substance added as a stabilizer, and label packages intended for direct use by the public to indicate that it is not intended for internal use.
- **USP REFERENCE STANDARDS (11)**
USP Mineral Oil RS

Minocycline for Injection

DEFINITION

Minocycline for Injection is sterile, freeze-dried Minocycline Hydrochloride suitable for parenteral use. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Mobile phase: Dimethylformamide, tetrahydrofuran, 0.2 M ammonium oxalate, and 0.01 M edetate disodium (120:80:600:180). Adjust with ammonium hydroxide to a pH of 7.2.

System suitability solution: Dissolve 10 mg of USP Minocycline Hydrochloride RS in 20 mL of 0.2 M ammonium oxalate. Heat on a water bath at 60° for 3 h, allow to cool, and dilute with water to 25.0 mL.

Standard solution: 0.5 mg/mL of minocycline from USP Minocycline Hydrochloride RS in water. Use this solution within 3 h.

Sample solution 1 (where it is represented as being in a single-dose container): Nominally 0.5 mg/mL of minocycline, prepared as follows. Constitute Minocycline for Injection in a volume of water, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a hypodermic needle and syringe, and dilute with water.

Sample solution 2 (where the label states the quantity of minocycline in a given volume of constituted solution): Nominally 0.5 mg/mL of minocycline, prepared as follows. Constitute Minocycline for Injection in a volume of water, corresponding to the volume of solvent specified in the labeling. Dilute a portion of constituted solution with water.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Samples: System suitability solution and Standard solution

[NOTE—The relative retention times for epiminocycline and minocycline are 0.7 and 1.0, respectively.]

Suitability requirements

Capacity factor: 5.0–11.5, Standard solution

Resolution: NLT 4.6 between epiminocycline and minocycline, System suitability solution

Tailing factor: 0.9–2.0 for minocycline, Standard solution

Relative standard deviation: NMT 2.0%, Standard solution

Analysis

Samples: Standard solution, and Sample solution 1 or Sample solution 2

Calculate the percentage of the labeled amount of minocycline (C₂₃H₂₇N₃O₇) in the container, or in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from Sample solution 1 or Sample solution 2

r_S = peak response from the Standard solution

C_S = concentration of USP Minocycline Hydrochloride RS in the Standard solution (mg/mL)

C_U = nominal concentration of Sample solution 1 or Sample solution 2 (mg/mL)

P = potency of minocycline in USP Minocycline Hydrochloride RS (μg/mg)

F = conversion factor, 0.001 mg/μg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

- UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

IMPURITIES**• LIMIT OF EPIMINOCYCLINE**

Mobile phase, System suitability solution, Standard solution, Sample solution 1 or Sample solution 2, Chromatographic system, and System suitability: Proceed as directed in the Assay.

[NOTE—The relative retention times for epiminocycline and minocycline are 0.7 and 1.0, respectively.]

Analysis: Calculate the percentage of epiminocycline in the portion of Minocycline for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of epiminocycline from Sample solution 1 or Sample solution 2

r_T = total area of all the peaks from Sample solution 1 or Sample solution 2

Acceptance criteria: NMT 6.0%

SPECIFIC TESTS**• pH (791)**

Sample solution: Nominally 10 mg/mL of minocycline

Acceptance criteria: 2.0–3.5

• WATER DETERMINATION, Method I (921)

Test preparation: Prepare as directed for a hygroscopic specimen.

Acceptance criteria: NMT 3.0%

- PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections

- STERILITY TESTS (71):** Meets the requirements

- BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 1.25 USP Endotoxin Units/mg of minocycline.

- CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for Injections and Implanted Drug Products (1), Specific Tests, Completeness and clarity of solutions.

- OTHER REQUIREMENTS:** It meets the requirements for Labeling (7), Labels and Labeling for Injectable Products.

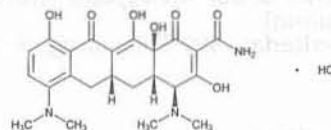
ADDITIONAL REQUIREMENTS**Change to read:**

- PACKAGING AND STORAGE:** Preserve as described in [•]Packaging and Storage Requirements (659), Injection Packaging, Packaging for constitution (CN 1-May-2017), protected from light.

- USP REFERENCE STANDARDS (11)**

USP Endotoxin RS

USP Minocycline Hydrochloride RS

Minocycline Hydrochloride

C₂₃H₂₇N₃O₇ · HCl 493.94
2-Naphthacenecarboxamide, 4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-, monohydrochloride, [4S-(4α,4α,5α,12α)]-; 4,7-Bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [13614-98-7].

DEFINITION

Minocycline Hydrochloride contains the equivalent of NLT 890 μg and NMT 950 μg of minocycline (C₂₃H₂₇N₃O₇) per mg, calculated on the anhydrous basis.

IDENTIFICATION

- **INFRARED ABSORPTION** (197K): Dry the *Standard* and *Sample* at 100° for 2 h before use.

ASSAY• **PROCEDURE**

[NOTE—Protect the *Standard solution* and *Sample solution* from light, store in a refrigerator, and use within 3 h.]

Mobile phase: Dimethylformamide, tetrahydrofuran, 0.2 M ammonium oxalate, and 0.01 M edetate disodium (120:80:600:180). Adjust with ammonium hydroxide to a pH of 7.2.

System suitability solution: Dissolve 10 mg of USP Minocycline Hydrochloride RS in 20 mL of 0.2 M ammonium oxalate. Heat on a water bath at 60° for 3 h, allow to cool, and dilute with water to 25.0 mL.

Standard solution: Equivalent to 500 µg/mL of minocycline (C₂₃H₂₇N₃O₇) from USP Minocycline Hydrochloride RS in water

Sample solution: Equivalent to 500 µg/mL of minocycline (C₂₃H₂₇N₃O₇) from Minocycline Hydrochloride in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for epiminocycline and minocycline are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.6 between epiminocycline and minocycline, *System suitability solution*

Tailing factor: 0.9–2.0 for the analyte peak, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of minocycline (C₂₃H₂₇N₃O₇) in the portion of Minocycline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of minocycline in the *Standard solution* (µg/mL)

C_U = concentration of the *Sample solution* (µg/mL)

P = potency of USP Minocycline Hydrochloride RS (µg/mg)

Acceptance criteria: 890–950 µg/mg on the anhydrous basis

IMPURITIES**Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.15%

Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 50 ppm (Official 1-Jan-2018)

Organic Impurities• **PROCEDURE**

Mobile phase, Standard solution, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*

[NOTE—Protect the *Standard solution* and the *Sample solutions* from light, store in a refrigerator, and use within 3 h.]

Sample solution 1: 0.25 mg/mL of Minocycline Hydrochloride

Sample solution 2: 5 µg/mL of Minocycline Hydrochloride in water

Sample solution 3: 3 µg/mL of Minocycline Hydrochloride in water

Run time: 2.6 times the retention time of minocycline, *Sample solution 1*

Analysis

Samples: *Sample solution 1*, *Sample solution 2*, and *Sample solution 3*

Calculate the percentage of epiminocycline in the portion of Minocycline Hydrochloride taken:

$$\text{Result} = (r_{E1}/r_{M3}) \times D_1 \times 100$$

r_{E1} = peak response of epiminocycline from *Sample solution 1*

r_{M3} = peak response of minocycline from *Sample solution 3*

D_1 = dilution factor for *Sample solution 3*
Calculate the total percentage of impurities other than epiminocycline in the portion of Minocycline Hydrochloride taken:

$$\text{Result} = (r_T/r_{M2}) \times D_2 \times 100$$

r_T = sum of peak responses of all impurities other than epiminocycline from *Sample solution 1*

r_{M2} = peak response of minocycline from *Sample solution 2*

D_2 = dilution factor for *Sample solution 2*

Acceptance criteria

Individual impurities: NMT 1.2% epiminocycline

Total impurities (excluding epiminocycline): NMT 2.0%

SPECIFIC TESTS

- **CRYSTALLINITY** (695): Meets the requirements
- **PH** (791): 3.5–4.5, in a solution equivalent to 10 mg/mL of minocycline
- **WATER DETERMINATION**, *Method I* (921): 4.3%–8.0%
- **STERILITY TESTS** (71): Where the label states that Minocycline Hydrochloride is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** (85): Where the label states that Minocycline Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 1.25 USP Endotoxin Units/mg of minocycline.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** (11)
USP Endotoxin RS
USP Minocycline Hydrochloride RS

Minocycline Hydrochloride Capsules**DEFINITION**

Minocycline Hydrochloride Capsules contain the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of minocycline (C₂₃H₂₇N₃O₇).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Mobile phase: Dimethylformamide, tetrahydrofuran, 0.2 M ammonium oxalate, and 0.01 M edetate disodium (120:80:600:180). Adjust with ammonium hydroxide to a pH of 7.2.

System suitability solution: Dissolve 10 mg of USP Minocycline Hydrochloride RS in 20 mL of 0.2 M ammonium oxalate. Heat on a water bath at 60° for 3 h, allow to cool, and dilute with water to 25.0 mL.

Standard solution: 0.5 mg/mL of minocycline from USP Minocycline Hydrochloride RS in water. Use this solution within 3 h.

Sample solution: Nominally 0.5 mg/mL of minocycline in water from combined contents of NLT 20 Capsules. Pass through a suitable filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for epiminocycline and minocycline are 0.7 and 1.0, respectively.]

Suitability requirements

Capacity factor: 5.0–11.5, *Standard solution*

Resolution: NLT 4.6 between epiminocycline and minocycline, *System suitability solution*

Tailing factor: 0.9–2.0 for minocycline, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Minocycline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

P = potency of minocycline in USP Minocycline Hydrochloride RS (μg/mg)

F = conversion factor, 0.001 mg/μg

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Detector: UV maximum at about 348 nm

Standard solution: USP Minocycline Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* (711). Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 12.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

- **USP REFERENCE STANDARDS (11)**

USP Minocycline Hydrochloride RS

Minocycline Hydrochloride Oral Suspension

DEFINITION

Minocycline Hydrochloride Oral Suspension contains the equivalent of NLT 90.0% and NMT 130.0% of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) and one or more suitable diluents, flavors, preservatives, and wetting agents in an aqueous vehicle.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Mobile phase: Dimethylformamide, tetrahydrofuran, 0.2 M ammonium oxalate, and 0.01 M edetate disodium (120:80:600:180). Adjust with ammonium hydroxide to a pH of 7.2.

System suitability solution: 2 mg/mL of USP Minocycline Hydrochloride RS in water. Transfer 5 mL of this solution to a small beaker, and heat on a steam bath for 60 min. Evaporate to dryness, and dissolve the residue in 25 mL of *Mobile phase*. Pass through a filter.

Standard solution: 0.5 mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Mobile phase*. Use the solution within 1 h.

Sample solution: Nominally 0.5 mg/mL of minocycline from Oral Suspension, freshly mixed and free from air bubbles, in *Mobile phase*. Use the solution within 1 h.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for epiminocycline and minocycline are 0.7 and 1.0, respectively.]

Suitability requirements

Capacity factor: 5.0–11.5, *Standard solution*

Resolution: NLT 4.6 between epiminocycline and minocycline, *System suitability solution*

Tailing factor: 0.9–2.0 for minocycline, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Minocycline Hydrochloride RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of minocycline in the *Sample solution* (mg/mL)
 P = potency of minocycline in USP Minocycline Hydrochloride RS ($\mu\text{g}/\text{mg}$)
 F = conversion factor, 0.001 mg/ μg
 Acceptance criteria: 90.0%–130.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905)
For single-unit containers
Acceptance criteria: Meets the requirements
- **DELIVERABLE VOLUME** (698): Meets the requirements

SPECIFIC TESTS

- **PH** (791): 7.0–9.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
USP Minocycline Hydrochloride RS

Minocycline Hydrochloride Tablets

DEFINITION

Minocycline Hydrochloride Tablets contain the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of minocycline ($\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_7$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY• **PROCEDURE**

Mobile phase: Dimethylformamide, tetrahydrofuran, 0.2 M ammonium oxalate, and 0.01 M edetate disodium (120:80:600:180). Adjust with ammonium hydroxide to a pH of 7.2.

System suitability solution: Dissolve 10 mg of USP Minocycline Hydrochloride RS in 20 mL of 0.2 M ammonium oxalate. Heat on a water bath at 60° for 3 h, allow to cool, and dilute with water to 25.0 mL.

Standard solution: 0.5 mg/mL of minocycline from USP Minocycline Hydrochloride RS in water. Use this solution within 3 h.

Sample solution: Nominally 0.5 mg/mL of minocycline from NLT 20 finely powdered Tablets in water. Shake for 1 min.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for epiminocycline and minocycline are 0.7 and 1.0, respectively.]

Suitability requirements

Capacity factor: 5.0–11.5, *Standard solution*

Resolution: NLT 4.6 between epiminocycline and minocycline, *System suitability solution*

Tailing factor: 0.9–2.0 for minocycline, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of minocycline ($\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Minocycline Hydrochloride RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of the *Sample solution* (mg/mL)
 P = potency of minocycline in USP Minocycline Hydrochloride RS ($\mu\text{g}/\text{mg}$)
 F = conversion factor, 0.001 mg/ μg
 Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Detector: UV maximum at about 348 nm

Standard solution: USP Minocycline Hydrochloride RS in *Medium*

Sample solution: Sample per *Dissolution* (711). Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of minocycline ($\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_7$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

SPECIFIC TESTS

- **WATER DETERMINATION, Method I** (921): NMT 12.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
USP Minocycline Hydrochloride RS

Minocycline Hydrochloride Extended-Release Tablets

DEFINITION

Minocycline Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of minocycline ($\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_7$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B.** The UV absorption spectrum of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the Assay.

ASSAY• **PROCEDURE**

Protect solutions containing minocycline from light.

Buffer: 3.5 g/L of tetrabutylammonium hydrogen sulfate, 2 g/L of anhydrous citric acid, and 6.8 g/L of monobasic potassium phosphate. Adjust with 10 N sodium hydroxide to a pH of 7.0.

Mobile phase: Acetonitrile and Buffer (24:76)

Diluent: Acetonitrile and water (20:80)

Standard solution: 0.045 mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Diluent*. Store at 4° and use within 24 h.

Sample stock solution: Nominally about 0.9 mg/mL of minocycline from Tablets prepared as follows. Transfer a suitable portion of finely powdered Tablets (NLT 10) to a suitable volumetric flask. Add acetonitrile, using 20% of the final volume, and mix vigorously for 15 min. Add water, using 65% of the final volume, and mix vigorously for 30 min. Dilute with water to volume and mix.

Sample solution: Nominally 0.045 mg/mL of minocycline from *Sample stock solution* in *Diluent*. Centrifuge and use the clear supernatant. Store at 4° and use within 24 h.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 277 nm. When this procedure is used for *Identification test B*, use a diode-array detector set at 200–400 nm.

Column: 4.6-mm × 15-cm; 5-μm packing L1

Temperatures

Column: 35°

Autosampler: 4°

Flow rate: 1.3 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Minocycline Hydrochloride RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of minocycline in the *Sample solution* (mg/mL)

P = potency of minocycline in USP Minocycline Hydrochloride RS (μg/mg)

F = conversion factor, 0.001 mg/μg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Protect solutions containing minocycline from light.

Medium: pH 6.8 phosphate buffer; 900 mL

Apparatus 2: 50 rpm

Times: 1, 2, and 5 h

Standard stock solution: 0.5 mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Medium*

Standard solution: ($L/900$) mg/mL of minocycline from *Standard stock solution* in *Medium*, where L is the label claim of minocycline in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

Mode: UV

Analytical wavelength: 348 nm

Cell: See Table 1.

Table 1

Tablet Strength (mg)	Cell Path Length (cm)
45	0.5
90	0.2
135	0.2

Blank: *Medium*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Autozero the instrument using the *Blank*.

Calculate the concentration (C_i) of minocycline ($C_{23}H_{27}N_3O_7$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result} = (A_u/A_s) \times C_s \times P \times F$$

A_u = absorbance of the *Sample solution* at time point i

A_s = absorbance of the *Standard solution*

C_s = concentration of the *Standard solution* (mg/mL)

P = potency of minocycline in USP Minocycline Hydrochloride RS (μg/mg)

F = conversion factor, 0.001 mg/μg

Calculate the percentage of the labeled amount (Q_i) of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_5)] + (C_1 \times V_5)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_5)]] + [(C_2 + C_1) \times V_5]\} \times (1/L) \times 100$$

C_i = concentration of minocycline in the portion of sample withdrawn at the specified time point (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_5 = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See Table 2.

Table 2

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	20–45
2	2	40–70
3	5	NLT 85

The percentages of the labeled amounts of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 2

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Protect solutions containing minocycline from light.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, and 4 h

Standard solution: 0.0225 mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Medium*

Sample solution: At the times specified, withdraw 10 mL of the solution under test and replace with 10 mL of *Medium*. Pass through a suitable filter. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV
 Analytical wavelength: 348 nm
 Cell: 1 cm
 Blank: Medium

Analysis

Samples: *Standard solution* and *Sample solution*
 Autozero the instrument using the *Blank*.
 Calculate the concentration (C_i) of minocycline ($C_{23}H_{27}N_3O_7$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result} = (A_U/A_S) \times C_S \times D \times P \times F$$

A_U = absorbance of the *Sample solution* at time point i
 A_S = absorbance of the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)
 D = dilution factor (mL/mL)
 P = potency of minocycline in USP Minocycline Hydrochloride RS ($\mu\text{g}/\text{mg}$)
 F = conversion factor, 0.001 mg/ μg
 Calculate the percentage of the labeled amount (Q_i) of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

C_i = concentration of minocycline in the portion of sample withdrawn at the specified time point (mg/mL)
 V = volume of *Medium*, 900 mL
 L = label claim (mg/Tablet)
 V_3 = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See Table 3.

Table 3

Time Point (i)	Time (h)	Amount Dissolved (%)	
		45 mg/Tablet	90 mg/Tablet and 135 mg/Tablet
1	1	40–60	40–60
2	2	70–95	70–90
3	4	NLT 85	NLT 85

The percentages of the labeled amounts of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at the times specified conform to *Dissolution* <711>, *Acceptance Table 2*.

Test 3

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Protect solutions containing minocycline from light.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Times: 0.5, 1.5, and 4 h

Standard solution: 0.021 mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Medium*

Sample solution: At the times specified, withdraw 10 mL of the solution under test and replace with 10 mL of *Medium*. Pass through a suitable filter. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV
 Analytical wavelength: 265 nm
 Cell: 1 cm
 Blank: Medium

Analysis

Samples: *Standard solution* and *Sample solution*
 Autozero the instrument using the *Blank*.
 Calculate the concentration (C_i) of minocycline ($C_{23}H_{27}N_3O_7$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result} = (A_U/A_S) \times C_S \times D \times P \times F$$

A_U = absorbance of the *Sample solution* at time point i
 A_S = absorbance of the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)
 D = dilution factor (mL/mL)
 P = potency of minocycline in USP Minocycline Hydrochloride RS ($\mu\text{g}/\text{mg}$)
 F = conversion factor, 0.001 mg/ μg
 Calculate the percentage of the labeled amount (Q_i) of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

C_i = concentration of minocycline in the portion of sample withdrawn at the specified time point (mg/mL)
 V = volume of *Medium*, 900 mL
 L = label claim (mg/Tablet)
 V_3 = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See Table 4.

Table 4

Time Point (i)	Time (h)	Amount Dissolved (%)
1	0.5	NMT 40
2	1.5	50–95
3	4	NLT 85

The percentages of the labeled amounts of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at the times specified conform to *Dissolution* <711>, *Acceptance Table 2*.

Test 4

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Protect solutions containing minocycline from light.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, and 4 h

Standard solution: ($L/900$) mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Medium*, where L is the label claim of minocycline in mg/Tablet

Sample solution: At the times specified, withdraw 5 mL of the solution under test and replace with 5 mL of *Medium*. Pass through a suitable filter. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 353 nm

Cell: 1 cm

Blank: Medium

Analysis**Samples:** Standard solution and Sample solution

Autozero the instrument using the Blank.

Calculate the concentration (C_i) of minocycline ($C_{23}H_{27}N_3O_7$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result} = (A_u/A_s) \times C_s \times D \times P \times F$$

 A_u = absorbance of the Sample solution at time point i A_s = absorbance of the Standard solution C_s = concentration of the Standard solution (mg/mL) D = dilution factor (mL/mL) P = potency of minocycline in USP Minocycline Hydrochloride RS ($\mu\text{g}/\text{mg}$) F = conversion factor, 0.001 mg/ μg Calculate the percentage of the labeled amount (Q_i) of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

 C_i = concentration of minocycline in the portion of sample withdrawn at the specified time point (mg/mL) V = volume of Medium, 900 mL L = label claim (mg/Tablet) V_3 = volume of the Sample solution withdrawn at each time point and replaced with Medium (mL)

Tolerances: See Table 5.

Table 5

Time Point (i)	Time (h)	Amount Dissolved (%)	
		45/Tablet and 90 mg/ Tablet	135 mg/ Tablet
1	1	35–50	35–50
2	2	63–78	67–82
3	4	NLT 90	NLT 90

The percentages of the labeled amounts of minocycline ($C_{23}H_{27}N_3O_7$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES• **ORGANIC IMPURITIES**

Protect solutions containing minocycline from light.

Buffer, Mobile phase, Diluent, and Sample solution:

Prepare as directed in the Assay.

Standard stock solution: Use the Standard solution as directed in the Assay.**Standard solution:** 0.009 mg/mL of minocycline from Standard stock solution in Diluent. Store at 4° and use within 24 h.**Sensitivity solution:** 0.9 $\mu\text{g}/\text{mL}$ of minocycline from Standard solution in Diluent. Store at 4° and use within 24 h.**System suitability solution:** Heat a portion of the Standard stock solution at 60° for about 2 h and cool. This solution contains a mixture of 4-epiminocycline and minocycline. Store at 4° and use within 24 h.**Chromatographic system:** Proceed as directed in the Assay, except use a flow rate of 1 mL/min.**System suitability****Samples:** Standard solution, Sensitivity solution, and System suitability solution**Suitability requirements****Resolution:** NLT 4.6 between minocycline and

4-epiminocycline, System suitability solution

Tailing factor: NMT 1.5, Standard solution**Relative standard deviation:** NMT 2.0%, Standard solution**Signal-to-noise ratio:** NLT 10, Sensitivity solution**Analysis****Samples:** Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

 r_u = peak response of each impurity from the Sample solution r_s = peak response of minocycline from the Standard solution C_s = concentration of USP Minocycline Hydrochloride RS in the Standard solution (mg/mL) C_u = nominal concentration of minocycline in the Sample solution (mg/mL) P = potency of minocycline in USP Minocycline Hydrochloride RS ($\mu\text{g}/\text{mg}$) F = conversion factor, 0.001 mg/ μg **Acceptance criteria:** See Table 6. The reporting threshold is 0.1%.**Table 6**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
4-Epiminocycline ^a	0.38	4.0
Desmethyl minocycline ^{b,c}	0.46	—
Sancycline ^{b,d}	0.68	—
5a,6-Anhydrominocycline ^{b,e}	0.81	—
Hydroxymethylminocycline ^{b,f}	0.92	—
Minocycline	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products ^g	—	2.0

^a (4R,4aS,5aR,12aS)-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.^b Process impurities are controlled in the drug substance and are not to be reported here. They are not included in total degradation products.^c (4R,4aS,5aR,12aS)-4-Dimethylamino-3,10,12,12a-tetrahydroxy-7-methylamino-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.^d 6-Desmethyl-6-deoxytetracycline; (4S,4aS,5aR,12aS)-4-Dimethylamino-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.^e (4S,4aS,12aS)-4,7-Bis(dimethylamino)-3,10,11,12a-tetrahydroxy-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide.^f (4S,4aS,5aR,12aS)-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-N-(hydroxymethyl)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.^g Total degradation products does not include 4-epiminocycline.**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Store in tightly closed containers at controlled room temperature.

- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if Test 1 is not used.
- **USP REFERENCE STANDARDS (11)**
USP Minocycline Hydrochloride RS

Minocycline Periodontal System

DEFINITION

Minocycline Periodontal System is an extended-release formulation of Minocycline Hydrochloride containing the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$).

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION (197U)**
Wavelength range: 250–450 nm
Standard stock solution: Transfer USP Minocycline Hydrochloride RS to a suitable volumetric flask. Dissolve first in dimethylformamide, using about 20% of the final volume, then dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.48 mg/mL of minocycline.
Standard solution: 0.024 mg/mL minocycline hydrochloride from *Standard stock solution* in water
Sample stock solution: Transfer Minocycline Periodontal System equivalent to 12 mg of minocycline hydrochloride to a 25-mL volumetric flask. Add 5.0 mL of dimethylformamide, and mix to dissolve. Dilute with water to volume and filter.
Sample solution: 0.024 mg/mL minocycline hydrochloride from *Sample stock solution* in water
Acceptance criteria: The *Sample solution* exhibits maxima at the same wavelengths as the *Standard solution*, concomitantly measured.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Mobile phase: Dimethylformamide, 0.2 M ammonium oxalate, and 0.1 M edetate disodium (25:55:20), adjusted with 0.4 M aqueous tetrabutylammonium hydroxide solution to a pH of 6.3 ± 0.2
Diluent: Dimethylformamide and methanol (1:1)
System suitability solution: Prepare a solution in water containing 2 mg/mL USP Minocycline Hydrochloride RS. Heat over a steam bath for 60 min. To one part of this solution add four parts of *Mobile phase* and mix. Refrigerate the solution immediately after preparation and during analysis, using a refrigerated autosampler.
Standard solution: 0.4 mg/mL of minocycline from USP Minocycline Hydrochloride RS in *Diluent*. Refrigerate the solution immediately after preparation and during analysis, using a refrigerated autosampler. [NOTE—Use low-actinic glassware.]
Sample solution: Mix the contents of NLT 10 dispensing units of Minocycline Periodontal System. Transfer a portion of the mixture, equivalent to 10 mg of minocycline, into a 25-mL volumetric flask. Add *Diluent* and sonicate for 2–5 min, or until the sample is dissolved. Dilute with *Diluent* to volume, and mix to obtain a solution having a nominal concentration of 0.4 mg/mL of minocycline, based on the label claim. Refrigerate the solution immediately after preparation and during analysis, using a refrigerated autosampler. [NOTE—Use low-actinic glassware.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC
Detector: UV 280 nm
Guard column: 4.6-mm \times 3-cm; 10- μ m packing L7
Column: 4.6-mm \times 15-cm; 5- μ m packing L7
Flow rate: 2 mL/min
Autosampler temperature: 5°
Injection size: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times of epiminocycline and minocycline are 0.81 and 1, respectively.]

Suitability requirements

Resolution: NLT 2.0 between epiminocycline and minocycline, *System suitability solution*

Tailing factor: NMT 2.0 for the minocycline peak, *System suitability solution*

Relative standard deviation: NMT 2.0% for the minocycline peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of minocycline ($C_{23}H_{27}N_3O_7$) in the portion of the Minocycline Periodontal System taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of minocycline from the *Sample solution*

r_S = peak response of minocycline from the *Standard solution*

C_S = concentration of USP Minocycline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of minocycline in the *Sample solution* (mg/mL)

P = potency of minocycline in USP Minocycline Hydrochloride RS (μ g/mg)

F = conversion factor, 0.001 mg/ μ g

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

DISSOLUTION

Medium: 6.9 g/L of monobasic sodium phosphate monohydrate in water, adjusted with phosphoric acid to a pH of 4.2. This solution is stable for 10 days.

Apparatus: Tube rotator. [NOTE—Suitable equipment is available as Labquake® tube rotator, catalog number 400110.]

0.1 M Edetate disodium: 37.2 g/L of edetate disodium in water

0.2 M Ammonium oxalate: 28.4 g/L of ammonium oxalate in water

Mobile phase: Mix 310 mL of 0.1 M *Edetate disodium* and 500 mL of 0.2 M *Ammonium oxalate*, adjust with 0.4 M aqueous tetrabutylammonium hydroxide to a pH of 6.2, and add 175 mL of dimethylformamide. The injector wash solution is a mixture of dimethylformamide and water (25:75).

Standard stock solution: 0.11 mg/mL of USP Minocycline Hydrochloride RS in *Medium*

Standard solutions: Dilute the *Standard stock solution* with *Medium* to obtain solutions with final concentrations of 0.088 mg/mL, 0.0528 mg/mL, 0.0352 mg/mL, 0.022 mg/mL, and 0.0176 mg/mL.

System suitability solution: Transfer 10 mg of USP Minocycline Hydrochloride RS to a 50-mL beaker. Add 5 mL of water and heat on a steam bath for 60 min. Add 20 mL of *Medium* or *Mobile phase*, and mix well. Store at 5°.

Sample solution: Use borosilicate glass tubes, 25 mm outside diameter and 15 cm long. Close the tubes with a snap type cell with a Teflon prong consisting of a

Teflon closure and holder that snap together, two 25- μ m stainless steel screens, two silicone gaskets, and a Teflon spacer (see Figure 1). Prepare six tubes as follows: partially assemble a release tube and tare its weight; dispense one dose of Minocycline Periodontal System into a partially assembled release cell (see Figure 1); record the sample weight in mg; assemble the cell so that the sample is enclosed between the two 25- μ m screens; close the cells and place each one of them into separate glass tubes containing 10 mL of *Medium* previously equilibrated at 37°; add the Teflon prong, and cap the tube with Teflon faced rubber-lined caps; seal with Teflon tape. Place the tubes in the tube rotator. Place the tube rotator in a convection incubator that is maintained at 37°. Allow the tubes to rotate for 4 h. Remove the solution under test, and add 10 mL of *Medium* previously equilibrated at 37°. Replace the tubes in the apparatus and rotate for 20 h (24 h total). Repeat the sampling procedure after 24 h (48 h total), and after another 24 h (72 h total).

Teflon Release Container

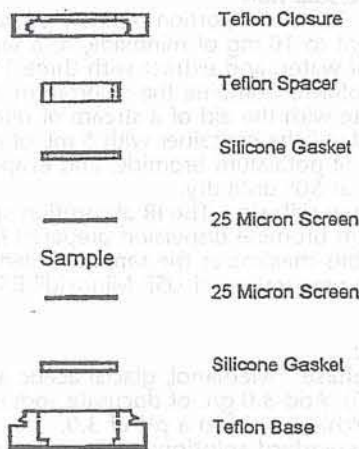


Figure 1. Sample Extraction Configuration

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Guard column: 4.6-mm \times 3-cm; 10- μ m packing L7

Column: 4.6-mm \times 3.3-cm; 5- μ m packing L1

Flow rate: 1.5 mL/min

Autosampler temperature: 5°

Injection size: 20 μ L for the 4 and 24 h time points;
50 μ L for the 48 and 72 h time points

Suitability requirements

Samples: *System suitability solution* and *Standard solutions*

Resolution: NLT 2.0 between epiminocycline and minocycline. Inject 20 μ L of the *System suitability solution*.

Tailing factor: NMT 2.0. Inject 20 μ L of the *System suitability solution*.

Relative standard deviation: NMT 2.0% for the minocycline peak, any of the *Standard solutions*

Analysis: Construct a calibration curve for each sampling interval by plotting the concentration of the *Standard solutions* versus peak area. Calculate the slopes and y-intercepts using linear regression analysis.

Calculate the release rate of minocycline:

$$\text{Result}_i = [(r_{ui} - y_i)/S_i] \times 10/(i \times W \times A)$$

- i = sampling time, 4, 24, 48, 72 h
- r_{ui} = peak response from each of the *Standard solutions* at time i
- y_i = y-intercept of the calibration curve at sampling time i
- S_i = slope of the calibration curve at sampling time i
- W = weight of the sample (mg)
- A = amount of minocycline in the sample (mg/mg of sample) as determined in the *Assay*

Tolerances

Time (h)	Release Rate (μ g/h) Average of 6 Measurements
0–4	NLT 25
4–24	NLT 1.0
24–48	NLT 0.2
48–72	NLT 0.05

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

IMPURITIES**Organic Impurities****• PROCEDURE**

Mobile phase, Diluent, *System suitability solution*, *Standard solution*, *Sample solution*, *Chromatographic system*, and *System suitability*: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each related compound in the portion of Minocycline Periodontal System taken:

$$\text{Result} = (r_u/r_T) \times 100$$

r_u = peak response of each impurity from the *Sample solution*

r_T = sum of the peak responses from the *Sample solution*. [NOTE—Exclude peaks eluting in the solvent front.]

Acceptance criteria

Individual impurities: NMT 6.0% of epiminocycline

Total impurities (excluding epiminocycline): NMT 3.5%

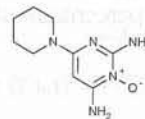
SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* (921): NMT 5.0%

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED ORGANISMS** (62): The total aerobic microbial count does not exceed 1000 cfu/g; the total combined molds and yeasts count does not exceed 100 cfu/g; and the product meets the requirements of the test for the absence of *Escherichia coli*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in a tight, light-resistant container. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
USP Minocycline Hydrochloride RS

MinoxidilC₉H₁₅N₅O

209.25

2,4-Pyrimidinediamine, 6-(1-piperidinyl)-, 3-oxide;
2,4-Diamino-6-piperidinopyrimidine 3-oxide [38304-91-5].

DEFINITION

Minoxidil contains NLT 97.0% and NMT 103.0% of minoxidil ($C_9H_{15}N_5O$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M): Do not dry specimens.

ASSAY• **PROCEDURE**

Mobile phase: Methanol, glacial acetic acid, and water (700:10:300). Add 3.0 g/L of docusate sodium. Adjust with perchloric acid to a pH of 3.0.

Internal standard solution: 0.2 mg/mL of medroxyprogesterone acetate in *Mobile phase*

Standard solution: 0.25 mg/mL of USP Minoxidil RS in *Internal standard solution*

Sample solution: 0.25 mg/mL of Minoxidil in *Internal standard solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm \times 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for the internal standard and minoxidil are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the internal standard and minoxidil

Relative standard deviation: NMT 2.0% from NLT four replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of minoxidil ($C_9H_{15}N_5O$) in the portion of Minoxidil taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of minoxidil to the internal standard from the *Sample solution*

R_S = peak response ratio of minoxidil to the internal standard from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.5%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-Jan-2018)

• **ORGANIC IMPURITIES**

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*.

Sample solution: 0.25 mg/mL of Minoxidil in *Mobile phase*

Analysis

Sample: *Sample solution*

Calculate the total percentage of impurities in the portion of Minoxidil taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = sum of the peak responses of all impurities from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: NMT 1.5%

SPECIFIC TESTS• **LOSS ON DRYING** (731)

Analysis: Dry a sample at 50° and at a pressure not exceeding 5 mm of mercury for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** (11)

USP Minoxidil RS

Minoxidil Tablets**DEFINITION**

Minoxidil Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of minoxidil ($C_9H_{15}N_5O$).

IDENTIFICATION• **INFRARED ABSORPTION**

Sample: Transfer a portion of finely powdered Tablets, equivalent to 10 mg of minoxidil, to a separator. Add 25 mL of water, and extract with three 15-mL portions of chloroform. Combine the chloroform extracts, and evaporate with the aid of a stream of nitrogen. Wash the inside of the container with 5 mL of alcohol, add 300 mg of potassium bromide, and evaporate under vacuum at 50° until dry.

Acceptance criteria: The IR absorption spectrum of the potassium bromide dispersion prepared from the *Sample* exhibits maxima at the same wavelengths as that of a similar preparation of USP Minoxidil RS.

ASSAY• **PROCEDURE**

Mobile phase: Methanol, glacial acetic acid, and water (70:1:30). Add 3.0 g/L of docusate sodium, and adjust with perchloric acid to a pH of 3.0.

Internal standard solution: 0.2 mg/mL of medroxyprogesterone acetate in *Mobile phase*

Standard solution: 0.25 mg/mL of USP Minoxidil RS in *Internal standard solution*

Sample solution: Nominally 0.25 mg/mL of minoxidil in *Internal standard solution*, prepared as follows.

Dissolve the equivalent to 5 mg of minoxidil, from powdered Tablets (NLT 10), in 20.0 mL of *Internal standard solution*, and shake for 5 min.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm \times 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for the internal standard and minoxidil are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the internal standard and minoxidil peaks

Relative standard deviation: NMT 2.0% from NLT four replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of minoxidil ($C_9H_{15}N_3O$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

- R_U = peak response ratio of minoxidil to the internal standard from the *Sample solution*
 R_S = peak response ratio of minoxidil to the internal standard from the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)
 C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: pH 7.2 Phosphate Buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 1: 75 rpm

Time: 15 min

Spectrometric conditions

Mode: UV

Detector: UV 231 nm for Tablets containing up to 10 mg of minoxidil, and UV 287 nm for Tablets containing more than 10 mg of minoxidil

Standard solution: USP Minoxidil RS in *Medium*

Sample solution: Sample per *Dissolution (711)*. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of minoxidil ($C_9H_{15}N_3O$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES• **ORGANIC IMPURITIES**

Diluent: Methanol and water (50:50)

Mobile phase: Dissolve 2.0 g of sodium lauryl sulfate in a mixture of methanol, glacial acetic acid, and water (60:1:40). Adjust with perchloric acid to a pH of 3.0 ± 0.1 , and pass through a suitable filter of 0.45- μ m pore size.

Standard solution: 5 μ g/mL of USP Minoxidil RS in *Diluent*

Sample solution: Nominally equivalent to 0.25 mg/mL of minoxidil in *Diluent* from powdered tablets (NLT 20). Initially add *Diluent* to about 60% of the volume of the flask, shake on a mechanical shaker for 20 min, and then dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm \times 15-cm; 3–10- μ m packing L1

Flow rate: 0.5 mL/min

Run time: NLT 3 times the retention time of the main peak

Injection volume: 40 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Sample solution* and *Standard solution*
Calculate the percentage of each individual impurity in the portion of the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_U/C_S) \times 100$$

- r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of minoxidil from the *Standard solution*

C_S = concentration of USP Minoxidil RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of minoxidil in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pyrimidine oxide analog ^a	0.19	—**
Minoxidil	1.00	—
Pyrimidine analog ^b	0.37	—**
Deoxominoxidil ^c	1.45	—**
Any other unknown impurity	—	0.2
Total impurities*	—	2.0

^a 2,4-Diamin-6-chloro-pyrimidine 3 oxide.

^b 2,4-Diamin-6-chloro-pyrimidine.

^c 2,4-Diamin-6-pipridino-pyrimidine.

* Total impurities is the sum of all the impurities, including process-related impurities. Disregard peaks less than 0.05%.

** A process-related impurity that is controlled in the drug substance.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Minoxidil RS

Minoxidil Topical Solution**DEFINITION**

Minoxidil Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of minoxidil ($C_9H_{15}N_3O$).

IDENTIFICATION• **A. INFRARED ABSORPTION (197M)**

Sample: Evaporate 1 mL of the Topical Solution under a stream of nitrogen while heating at 50°.

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: Add 0.65 mL of heptafluorobutyric acid to a 1000-mL volumetric flask. Dilute with water to volume.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
10.0	60	40
10.1	100	0
15.0	100	0

Diluent: Methanol and water (50:50)

System suitability solution: 0.4 mg/mL of USP Minoxidil RS and 0.001 mg/mL of USP Minoxidil Related Compound E RS in *Diluent*

Standard solution: 0.05 mg/mL of USP Minoxidil RS in *Diluent*

Sample solution: Nominally 0.05 mg/mL of minoxidil in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 2.1-mm × 10-cm; 1.7-μm packing L1

Column temperature: 35°

Flow rate: 0.4 mL/min

Injection volume: 1 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between minoxidil and minoxidil related compound E, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of minoxidil (C₉H₁₅N₅O) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Minoxidil RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of minoxidil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• ORGANIC IMPURITIES

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.4 μg/mL of USP Minoxidil RS in *Diluent*

Sample solution: Nominally 0.4 mg/mL of minoxidil in *Diluent*. Pass through a suitable filter of 0.2-μm pore size.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between minoxidil and minoxidil related compound E, *System suitability solution*

Relative standard deviation: NMT 2.8%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each unspecified impurity in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response of minoxidil from the *Standard solution*

C_S = concentration of USP Minoxidil RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of minoxidil in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Minoxidil related compound A (pyrimidine oxide analog) ^{a,b}	0.36	—
Pyrimidine analog ^{b,c}	0.51	—
Minoxidil	1.00	—
Minoxidil related compound E (deoxyminoxidil) ^{b,d}	1.03	—
Individual unspecified impurity	—	0.2
Total impurities	—	2.0

^a 2,6-Diamino-4-chloropyrimidine 1-oxide.

^b Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

^c 6-Chloropyrimidine-2,4-diamine.

^d 6-(Piperidin-1-yl)pyrimidine-2,4-diamine.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

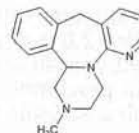
USP Minoxidil RS

USP Minoxidil Related Compound E RS

6-(Piperidin-1-yl)pyrimidine-2,4-diamine.

C₉H₁₅N₅ 193.25

Mirtazapine



C₁₇H₁₉N₃ 265.35
 Pyrazino[2,1-a]pyrido[2,3-c][2]benzazepine, 1,2,3,4,10,14b-hexahydro-2-methyl-;
 1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-a]pyrido[2,3-c][2]-benzazepine [85650-52-8].

DEFINITION

Mirtazapine contains NLT 98.0% and NMT 102.0% of mirtazapine (C₁₇H₁₉N₃), calculated on the anhydrous basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION (197K)**

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Diluent: Acetonitrile and water (1:1)

Buffer: Dissolve 18 g of tetramethylammonium hydroxide pentahydrate in 950 mL of water. Adjust with phosphoric acid to a pH of 7.4. Dilute with water to 1 L.

Mobile phase: Acetonitrile, methanol, tetrahydrofuran, and *Buffer* (15: 12.5: 7.5: 65)

Standard solution: 0.3 mg/mL of USP Mirtazapine RS in *Diluent*

Sample solution: 0.3 mg/mL of Mirtazapine in *Diluent*
Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 7000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mirtazapine (C₁₇H₁₉N₃) in the portion of Mirtazapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL)

C_U = concentration of Mirtazapine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 µg/g • (Official 1-Jan-2018)

ORGANIC IMPURITIES

Diluent, Buffer, and Mobile phase: Prepare as directed in the *Assay*.

System suitability solution: 1.5 mg/mL of USP Mirtazapine Resolution Mixture RS in *Diluent*

Standard solution: 0.0015 mg/mL of USP Mirtazapine RS in *Diluent*

Sample solution: 1.5 mg/mL of Mirtazapine in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: Twice the retention time of mirtazapine

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are listed in *Table 1*.]

Suitability requirements

Resolution: NLT 1.5 between acyclomirtazapine methyl derivative (impurity E) and 10-ketomirtazapine (impurity F), *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Mirtazapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any impurity from the *Sample solution*

r_S = peak response of mirtazapine from the *Standard solution*

C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL)

C_U = concentration of Mirtazapine in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

[NOTE—Disregard any peak with a result of 0.05% or less, as calculated using the formula given above.]

Acceptance criteria: See *Table 1*.

Table 1

Impurity Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mirtazapine N-oxide ^a	0.2	0.8	0.1
Acyclomirtazapine alcohol ^b	0.3	0.8	0.1
1-Ketomirtazapine ^c	0.35	1.0	0.1
Desmethylmirtazapine ^d	0.4	1.0	0.1
Mirtazapine	1.0	—	—
Acyclomirtazapine methyl derivative ^e	1.3	1.0	0.1
10-Ketomirtazapine ^f	1.35	5.0	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[NOTE—Disregard any peak representing less than 0.05% of the main peak and any peak that is due to the *Diluent*.]

^a 1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine 2-oxide (Impurity A).

^b (2-(4-Methyl-2-phenylpiperazin-1-yl)pyridin-3-yl)methanol (Impurity B).

^c (2-Methyl-3,4,10,14b-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-1(2*H*)-one (Impurity C).

^d 1,2,3,4,10,14b-Hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine (Impurity D).

^e 4-Methyl-1-(3-methylpyridin-2-yl)-2-phenylpiperazine (Impurity E).

^f 2-Methyl-1,2,3,4-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-10(14*bH*)-one (Impurity F).

SPECIFIC TESTS

- **WATER DETERMINATION, Method I** (921): NMT 3.5%

- **OPTICAL ROTATION, Specific Rotation** (781S)

Sample solution: 10 mg/mL, in denatured alcohol

Acceptance criteria: +2° to −2°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label it to indicate whether it is anhydrous or hemihydrate.
- **USP REFERENCE STANDARDS** (11)
 - USP Mirtazapine RS
 - USP Mirtazapine Resolution Mixture RS
 - This resolution mixture contains approximately 0.1% w/w each of the following:
 - Impurity A: 1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine 2-oxide.
 - Impurity B: (2-(4-Methyl-2-phenylpiperazin-1-yl)pyridin-3-yl)methanol.
 - Impurity C: (2-Methyl-3,4,10,14b-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-1(2*H*)-one.
 - Impurity D: 1,2,3,4,10,14b-Hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine.
 - Impurity E: 4-Methyl-1-(3-methylpyridin-2-yl)-2-phenylpiperazine.
 - Impurity F: 2-Methyl-1,2,3,4-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-10(14*bH*)-one.

Mirtazapine Tablets

DEFINITION

Mirtazapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

Extraction mixture: *n*-Hexane and water (1:1)

Sample: Transfer an amount equivalent to 30 mg of mirtazapine from finely powdered Tablets to a suitable centrifuge tube. Add *Extraction mixture* to obtain a solution of 1 mg/mL of mirtazapine in *n*-hexane. Shake for 5 min, and centrifuge. Decant, and evaporate the supernatant.

Standard: Dissolve USP Mirtazapine RS in *Extraction mixture* to obtain a solution having a concentration of about 1 mg/mL of mirtazapine in *n*-hexane. Shake for 5 min, and centrifuge. Decant, and evaporate the supernatant.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Diluent: Acetonitrile and water (1:1)

Buffer: Dissolve 18.0 g of tetramethylammonium hydroxide pentahydrate in 950 mL of water. Adjust with phosphoric acid to a pH of 7.4, and dilute with water to 1 L.

Mobile phase: Acetonitrile, methanol, tetrahydrofuran, and *Buffer* (15:12.5:7.5:65)

Standard solution: 0.3 mg/mL of USP Mirtazapine RS in *Diluent*

Sample solution: Nominally 0.3 mg/mL of mirtazapine (from an amount equivalent to the weight of 1 Tablet from NLT 20 finely powdered Tablets) in *Diluent*. Shake vigorously for 10 min, centrifuge an aliquot, and use the clear supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 7000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 15 min

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

Standard solution: USP Mirtazapine RS in *Medium* in a concentration similar to the one expected in the *Sample solution*

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 315 nm

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Diluent, Buffer, and Mobile phase: Proceed as directed in the *Assay*.

System suitability solution: 1.5 mg/mL of USP Mirtazapine Resolution Mixture RS in *Diluent*

Standard solution: 0.015 mg/mL of USP Mirtazapine RS in *Diluent*

Sample solution: 1.5 mg/mL of mirtazapine (from an amount equivalent to the weight of 1 Tablet from NLT 20 finely powdered Tablets) in *Diluent*. Shake vigorously for 10 min, centrifuge an aliquot, and use the clear supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: 2 times the retention time of mirtazapine

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are listed in *Table 1*.]

Suitability requirements

Resolution: NLT 1.5 between acyclomirtazapine methyl derivative (impurity E) and 10-ketomirtazapine (impurity F), *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- r_U = peak response of any impurity from the *Sample solution*
 r_S = mirtazapine peak response from the *Standard solution*
 C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of the *Sample solution* (mg/mL)
 F = relative response factor for the corresponding impurity (see Table 1)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mirtazapine <i>N</i> -oxide ^a	0.2	0.8	0.2
Acyclomirtazapine alcohol ^{b,g}	0.3	—	—
1-Ketomirtazapine ^c	0.35	1.0	0.2
Desmethylmirtazapine ^{d,g}	0.4	—	—
Mirtazapine	1.0	—	—
Acyclomirtazapine methyl derivative ^{e,g}	1.3	—	—
10-Ketomirtazapine ^f	1.35	5.0	0.2
Any individual unspecified degradation product	—	1.0	0.2
Total impurities	—	—	2.0

[NOTE—Disregard any peak representing less than 0.05% of the main peak and any peak that is due to the *Diluent*.]

^a 1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-*a*]pyrido[2,3-*c*] [2]benzazepine 2-oxide. (Impurity A)

^b (2-(4-Methyl-2-phenylpiperazin-1-yl)pyridin-3-yl)methanol. (Impurity B)

^c (2-Methyl-3,4,10,14b-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-1(2*H*)-one. (Impurity C)

^d 1,2,3,4,10,14b-Hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*] [2]benzazepine. (Impurity D)

^e 4-Methyl-1-(3-methylpyridin-2-yl)-2-phenylpiperazine. (Impurity E)

^f 2-Methyl-1,2,3,4-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-10(14*bH*)-one. (Impurity F)

^g Process impurity. Included for identification purposes only. Not to be included in *Total impurities*.

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
 - USP Mirtazapine RS
 - USP Mirtazapine Resolution Mixture RS

This resolution mixture contains approximately 0.1% w/w of each of the following:

Impurity A: 1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-*a*]pyrido[2,3-*c*] [2]benzazepine 2-oxide.

Impurity B: (2-(4-Methyl-2-phenylpiperazin-1-yl)pyridin-3-yl)methanol.

Impurity C: (2-Methyl-3,4,10,14b-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-1(2*H*)-one.

Impurity D: 1,2,3,4,10,14b-Hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*] [2]benzazepine.

Impurity E: 4-Methyl-1-(3-methylpyridin-2-yl)-2-phenylpiperazine.

Impurity F: 2-Methyl-1,2,3,4-tetrahydrobenzo[*c*]pyrazino[1,2-*a*]pyrido[3,2-*f*]azepin-10(14*bH*)-one.

Mirtazapine Orally Disintegrating Tablets

DEFINITION

Mirtazapine Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of mirtazapine ($C_{17}H_{19}N_3$).

IDENTIFICATION

A. INFRARED ABSORPTION (197K)

Standard solution: Dissolve 30 mg of USP Mirtazapine RS in a separatory funnel containing 30 mL of water, and add 30 mL of *n*-hexane. Shake vigorously for 5 min. Allow the solution to separate into two layers. Filter the *n*-hexane layer through glass wool, and evaporate to dryness.

Sample solution: Transfer a quantity of finely powdered Tablets, equivalent to 30 mg of mirtazapine, to a separatory funnel. Add 30 mL of water and 30 mL of *n*-hexane. Shake vigorously for 5 min. Allow the solution to separate into two layers. Filter the *n*-hexane layer through glass wool, and evaporate to dryness.

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Diluent: Acetonitrile and water (50:50)

Diluted phosphoric acid: Water and phosphoric acid (1000:3)

Buffer: Dissolve 1 g of monobasic potassium phosphate and 1.7 g of pentanesulfonic acid sodium salt in 1 L of water. Adjust with *Diluted phosphoric acid* to a pH of 4.7 ± 0.1, and filter.

Mobile phase: Acetonitrile and *Buffer* (25:75)

Standard stock solution: 0.3 mg/mL of USP

Mirtazapine RS in *Diluent*

Standard solution: 0.036 mg/mL of USP Mirtazapine RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: 0.3 mg/mL of mirtazapine in *Diluent* (from NLT 20 Tablets, finely powdered). Sonicate for 15 min with occasional swirling, and shake for 30 min. [NOTE—Alternatively, dissolve 10 Tablets in a volume of a mixture of acetonitrile and water (90:10) to obtain a 0.3 mg/mL solution of mirtazapine. Shake or stir until the mixture is free from lumps.]

Sample solution: Nominally, 0.036 mg/mL of mirtazapine in *Mobile phase* obtained as follows: transfer 40 mL of the *Sample stock solution* into a centrifuge tube, and centrifuge at 3000 rpm for 10 min. Transfer 6.0 mL of the supernatant into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass the portion through a polypropylene membrane filter of 0.45-μm pore size. Discard at least the first 5 mL of filtrate.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection size: 20 μL

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of mirtazapine (C₁₇H₁₉N₃) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Mirtazapine RS (mg/mL) C_U = nominal concentration of mirtazapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISINTEGRATION** (701): NMT 60 s

- **DISSOLUTION** (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 15 min

Sample solution: Sample per *Dissolution* (711). Pass through a filter of 0.45-μm pore size, and discard the first 5 mL of the filtrate.Standard solution: 33 μg/mL of USP Mirtazapine RS in *Medium***Instrumental conditions**(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 316 nm

Blank: *Medium*

Cell: 0.5 cm

Analysis: Determine the percentage of mirtazapine (C₁₇H₁₉N₃) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of USP Mirtazapine RS in the *Standard solution* (mg/mL) V = volume, 900 mL L = label claim of mirtazapine (mg/Tablet)Tolerances: NLT 80% (Q) of the labeled amount of mirtazapine (C₁₇H₁₉N₃) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A: Dissolve 7.2 g of tetramethylammonium hydroxide pentahydrate in 4 L of water. Add 1 mL of triethylamine. Adjust with phosphoric acid to a pH of 7.4.

Solution B: Acetonitrile, methanol, and tetrahydrofuran (170:145:85)

Diluent: Acetonitrile and water (50:50)

Mobile phase: See *Table 1*.**Table 1** (Continued)

Time (min)	Solution A (%)	Solution B (%)
10.0	46	54
18.4	46	54
18.5	61	39
22.0	61	39

System suitability solution: 0.3 mg/mL of USP Mirtazapine RS in *Diluent*

Standard solution: 0.015 mg/mL each of USP

Mirtazapine RS, USP Mirtazapine Related Compound A

RS, USP Mirtazapine Related Compound B RS, USP

Mirtazapine Related Compound C RS, and USP

Mirtazapine Related Compound D RS in *Diluent*Sample solution: Nominally, 1.5 mg/mL of mirtazapine in *Diluent* from NLT 5 Tablets**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection size: 10 μL

System suitabilitySamples: *System suitability solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0, *System suitability solution*Relative standard deviation: NMT 2.0%, *System suitability solution*Resolution: NLT 4.0 between the mirtazapine and mirtazapine related compound D peaks, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual specified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of each individual specified impurity from the *Sample solution* r_S = peak response of the corresponding related compound from the *Standard solution* C_S = concentration of each individual impurity in the *Standard solution* (mg/mL) C_U = nominal concentration of mirtazapine in the *Sample solution* (mg/mL)

Calculate the percentage of each individual unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of each individual impurity from the *Sample solution* r_S = peak response of mirtazapine from the *Standard solution* C_S = concentration of mirtazapine in the *Standard solution* (mg/mL) C_U = nominal concentration of mirtazapine in the *Sample solution* (mg/mL)Acceptance criteria: See *Table 2*. [NOTE—Disregard any peak less than 0.05%.]**Table 1**

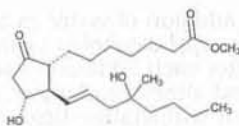
Time (min)	Solution A (%)	Solution B (%)
0	61	39
6.0	61	39

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mirtazapine related compound B	0.23	0.5
Mirtazapine related compound C	0.51	0.5
Mirtazapine related compound A	0.62	0.5
Mirtazapine	1.0	—
Mirtazapine related compound D	1.3	0.5
Any individual unspecified degradation product	—	0.5
Total impurities	—	3.0

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Store at controlled room temperature. Protect from light and moisture.
- USP REFERENCE STANDARDS (11)**
 - USP Mirtazapine RS
 - USP Mirtazapine Related Compound A RS
1,2,3,4,10,14b-Hexahydropyrazino[2,1-a]pyrido[2,3-c][2]benzazepine.
 $C_{16}H_{17}N_3$ 251.33
 - USP Mirtazapine Related Compound B RS
1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-a]pyrido[2,3-c][2]benzazepine 2-oxide monohydrate.
 $C_{17}H_{19}N_3O \cdot H_2O$ 299.36
 - USP Mirtazapine Related Compound C RS
2-Methyl-3,4,10,14b-tetrahydrobenzo[c]pyrazino[1,2-a]pyrido[3,2-f]azepin-1(2H)-one.
 $C_{17}H_{17}N_3O$ 279.34
 - USP Mirtazapine Related Compound D RS
2-Methyl-1,2,3,4-tetrahydrobenzo[c]pyrazino[1,2-a]pyrido[3,2-f]azepin-10(14bH)-one.
 $C_{17}H_{17}N_3O$ 279.34

Misoprostol

$C_{22}H_{38}O_5$ 382.53
 Prost-13-en-1-oic acid, 11,16-dihydroxy-16-methyl-9-oxo-, methyl ester, (1*R**,2*R**,3*R**,*E*)-;
 (±)-Methyl (1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(4*R**S*)-4-hydroxy-4-methyl-1-octenyl]-5-oxocyclopentaneheptanoate [59122-46-2].

DEFINITION

Misoprostol contains NLT 97.0% and NMT 102.0% of $C_{22}H_{38}O_5$, calculated on the anhydrous basis.

IDENTIFICATION

- A. INFRARED ABSORPTION (1975)**
 Sample solution: 30 mg/mL
 Medium: Chloroform
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY**PROCEDURE**

Mobile phase: 2,2,4-Trimethylpentane, dioxane, and acetonitrile (78:21.5:0.5)

Standard solution: 5.0 mg/mL of USP Misoprostol RS in *Mobile phase*

Sample solution: 5.0 mg/mL of Misoprostol in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-μm packing L3

Flow rate: 2 mL/min

Injection size: 20 μL

System suitability

Sample: *Standard solution*

[NOTE—Identify the impurities based on the retention times shown in *Impurity Table 1*.]

Suitability requirements

Resolution: NLT 1.2, between the second diastereomer peak for 12-epimisoprostol and the Misoprostol peak

Relative standard deviation: NMT 1.0%, for three replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{22}H_{38}O_5$ in the portion of Misoprostol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the *Sample solution*

r_S = peak response of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES**Organic Impurities****PROCEDURE 1**

Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability:

Proceed as directed in the Assay.

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatogram for at least 3 times the retention time of the Misoprostol peak, and measure the peak responses. Identify the impurities based on the retention times shown in *Impurity Table 1*.

Calculate the percentage of each impurity in the portion of Misoprostol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of the *Standard solution*

C_S = concentration of USP Misoprostol RS in the *Standard solution* (mg/mL)

C_U = concentration of Misoprostol in the *Sample solution* (mg/mL)

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 1.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
A-Type misoprostol ^a	0.22	7.8	0.1
B-Type misoprostol ^b	0.33	0.80	0.1
Norprostil ^c	0.51	8.4	0.1
8-Epimisoprostol ^d	0.71	1.05	0.3
12-Epimisoprostol ^e	0.86 and 0.92 ^f	1.08	1.0 ^f
Misoprostol	1.0	—	—
Any other individual impurity	—	1.0	0.1

^a Methyl 7-[(1*R**,2*S**)-2-[(*E*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopent-3-enyl]heptanoate.

^b (*E*)-Methyl 7-[2-(4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopent-1-enyl]heptanoate.

^c Methyl 7-(3-hydroxy-5-oxocyclopent-1-enyl)heptanoate.

^d Methyl (1*S**,2*R**,3*R**)-3-hydroxy-2-[(*E*)-4-hydroxy-4-methyl-1-octenyl]-5-oxocyclopentaneheptanoate.

^e Methyl (1*S**,2*R**,3*S**)-3-hydroxy-2-[(*E*)-4-hydroxy-4-methyl-1-octenyl]-5-oxocyclopentaneheptanoate.

^f 12-Epimisoprostol consists of two diastereomers that are separated under these conditions; integrate both peaks together for the impurity calculations.

• PROCEDURE 2: CONTENT OF DIASTEREOMERS

Mobile phase: Hexane, ethanol, and isopropyl alcohol (94:4:2)

Sample solution: 1.0 mg/mL of Misoprostol in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 25-cm; 5-μm packing L3

Column temperature: 40°

Flow rate: 1 mL/min

Injection size: 20 μL

System suitability

Sample: *Sample solution*

[NOTE—Identify the components based on their relative retention times which are about 0.92 for the first diastereomer peak and 1.0 for the second diastereomer peak.]

Suitability requirements

Resolution: NLT 2.0, between the two diastereomer peaks

Relative standard deviation: NMT 2.0% from the area of the first diastereomer peak

Analysis

Sample: *Sample solution*

Calculate the fraction of the first diastereomer in the portion of Misoprostol taken:

$$\text{Result} = r_1/(r_1 + r_2)$$

r_1 = peak response for the first diastereomer

r_2 = peak response for the second diastereomer

Acceptance criteria

Fraction of the first diastereomer: 0.51–0.56

SPECIFIC TESTS

- **WATER DETERMINATION, Method 1c (921):** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a freezer.

• USP REFERENCE STANDARDS (11)

USP Misoprostol RS

Misoprostol Dispersion

DEFINITION

Misoprostol Dispersion is a mixture of Misoprostol and Hypromellose. It contains NLT 95.0% and NMT 104.0% of the labeled amount of misoprostol ($C_{22}H_{38}O_5$).

IDENTIFICATION

• A. ULTRAVIOLET ABSORPTION

Perform both *Procedure 1* and *Procedure 2*.

Procedure 1

Medium: Methanol and water (4:1)

Sample solution: Nominally 16 μg/mL of misoprostol in *Medium* prepared as follows. Dissolve the amount of Misoprostol Dispersion, equivalent to 400 μg of misoprostol, in 25 mL of *Medium*.

Blank: Prepare a solution of hypromellose in *Medium* having the same concentration as in the *Sample solution*.

Analysis: Determine UV absorption spectrum of *Sample solution* against the *Blank* from 330–230 nm.

Acceptance criteria 1: It exhibits no maximum near 280 nm.

Procedure 2

Medium: Methanol and 1 N potassium hydroxide (4:1)

Sample solution: Add 10 mL of *Medium* to 10 mL of the *Sample solution* prepared from *Procedure 1*. Allow to stand for 30 min at room temperature.

Blank: Add 10 mL of *Medium* to 10 mL of the *Blank* prepared from *Procedure 1*. Allow to stand for 30 min at room temperature.

Analysis: Determine UV absorption spectrum of *Sample solution* against the *Blank* from 330–230 nm.

Acceptance criteria 2: It exhibits a maximum near 280 nm.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

[NOTE—During addition of water to a solution of misoprostol in isopropyl alcohol, an exothermic reaction takes place. After each addition of water, invert the flask to mix isopropyl alcohol and water. Allow the solution to cool to room temperature before the final dilution.]

Buffer: 1.36 g/L of monobasic potassium phosphate in water, adjusted with phosphoric acid to a pH of 3.0 ± 0.1

Mobile phase: Isopropyl alcohol and *Buffer* (27:73)

Standard stock solution: 0.5 mg/mL of USP Misoprostol RS in isopropyl alcohol. [NOTE—This solution is stable up to 28 days when stored at 5 ± 3°.]

Standard solution: 0.1 mg/mL of USP Misoprostol RS in water from *Standard stock solution*. [NOTE—This solution is stable up to 7 days when stored at 5 ± 3°.]

Sample solution: Nominally 0.1 mg/mL of misoprostol prepared as follows. Place an amount of Misoprostol Dispersion, equivalent to about 10 mg of misoprostol, into a 100-mL volumetric flask, and add 25 mL of isopropyl alcohol. Shake to disperse the solid, place the solution in an ice bath, swirl, and allow to cool for 10 min. Carefully add about 70 mL of water, previously chilled in a refrigerator, remove from the ice bath, and shake. Sonicate as necessary at a temperature not exceeding 20°. Equilibrate to room temperature, dilute with water to volume, and immediately cool the solu-

tion to 5°. [NOTE—This solution is stable up to 7 days when stored at 5 ± 3°.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Temperatures

Column: 50 ± 2°; the *Mobile phase* must be preheated prior to introduction on the column.

Autosampler: 5 ± 3°

Flow rate: 1.5 mL/min

Injection volume: 100 μL

System suitability

Sample: *Standard solution*

[NOTE—USP Misoprostol RS contains 12-epimisoprostol as a minor component. The relative retention times for 12-epimisoprostol and misoprostol are 0.84 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.7 between 12-epimisoprostol and misoprostol

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of misoprostol (C₂₂H₃₈O₅) in the portion of Misoprostol Dispersion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Misoprostol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of misoprostol in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–104.0%

IMPURITIES

• ORGANIC IMPURITIES

[NOTE—During the addition of water to a solution of misoprostol in isopropyl alcohol, an exothermic reaction takes place. After each addition of water, invert the flask to mix isopropyl alcohol and water. Allow the solution to cool to room temperature before the final dilution.]

Buffer, *Mobile phase*, *Standard solution*, *Sample solution*, and *Chromatographic system*: Proceed as directed in the *Assay*.

Diluent: Isopropyl alcohol and water (27:73)

Blank: Prepare a solution of hypromellose in the *Diluent* having the same concentration as in the *Sample solution*.

Diluted standard solution: 0.5 μg/mL of USP Misoprostol RS in *Diluent* from *Standard solution*

Sensitivity solution: 0.1 μg/mL of USP Misoprostol RS in *Diluent* from *Diluted standard solution*

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.7 between 12-epimisoprostol and misoprostol, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Sample solution*, *Blank*, and *Diluted standard solution*

Calculate the percentage of any individual impurity in the portion of Misoprostol Dispersion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of misoprostol from the *Diluted standard solution*

C_S = concentration of USP Misoprostol RS in the *Diluted standard solution* (μg/mL)

C_U = nominal concentration of misoprostol in the *Sample solution* (μg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard a peak from the *Diluent*, eluting at about 4 min, and any other peak observed in the *Blank*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
12-Epimisoprostol	0.84	—	— ^a
8-Epimisoprostol ^b	0.90	0.90	0.50
Misoprostol	1.0	—	—
B-Type misoprostol ^c	1.6	0.78	0.30
A-Type misoprostol ^d	1.9	2.6	0.50
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	1.8

^a This is a process impurity in the manufacturing of misoprostol. It is controlled in the misoprostol, which is the starting material for the Misoprostol Dispersion. This impurity is included in the table for identification only, and it is not to be reported or included in the total impurities for the Misoprostol Dispersion.

^b Methyl (1*S**,2*R**,3*R**)-3-hydroxy-2-[(*E*)-4-hydroxy-4-methyl-1-octenyl]-5-oxocyclopentaneheptanoate.

^c (*E*)-Methyl 7-[2-(4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopent-1-enyl]heptanoate.

^d Methyl 7-[(1*R**,2*S**)-2-[(*E*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopent-3-enyl]heptanoate.

SPECIFIC TESTS

• WATER DETERMINATION, *Method 1c* (921)

Sample: 300 mg

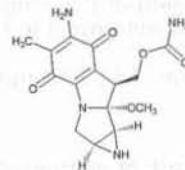
Analysis: Perform the test immediately after opening the sample container. Use the evaporation technique in which water is released and evaporated from the *Sample* by heating it in an external oven at 105° and transferring it to the reaction cell with the aid of an inert gas.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and store in a refrigerator.
- **LABELING:** The label states that this article is not intended for direct administration to humans or animals. Label it to indicate the nominal concentration or percentage of misoprostol in the Misoprostol Dispersion.
- **USP REFERENCE STANDARDS (11)**
USP Misoprostol RS

Mitomycin



C₁₅H₁₈N₄O₅ 334.33
Azirino[2',3':3,4]pyrrolo[1,2-*a*]indole-4,7-dione, 6-amino-8-[[[aminocarbonyloxy]methyl]-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-5-methyl-, [1*aS*-(1*α*,8*β*,8*α*,8*β*)]-];

(1aS,8S,8aR,8bS)-(6-Amino-8a-methoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2',3':3,4]pyrrolo[1,2-a]indol-8-yl)methyl carbamate;
Mitomycin C [50-07-7].

DEFINITION

Mitomycin has a potency of NLT 970 µg/mg of mitomycin (C₁₅H₁₈N₄O₅).

IDENTIFICATION

• A. INFRARED ABSORPTION (197M)

Analysis: Do not dry the sample and standard.

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Mobile phase: Dissolve 1.54 g of ammonium acetate in 250 mL of methanol. Add 5.0 mL of 0.83 N acetic acid and water to make 1000 mL.

System suitability solution: 0.5 mg/mL of USP Mitomycin RS and 7.5 mg/mL of 3-ethoxy-4-hydroxybenzaldehyde in *N,N*-dimethylacetamide

Standard solution: 0.5 mg/mL of USP Mitomycin RS in *N,N*-dimethylacetamide

Sample solution: 0.5 mg/mL of Mitomycin in *N,N*-dimethylacetamide

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 3.9-mm × 30-cm; 10-µm packing L11

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for mitomycin and 3-ethoxy-4-hydroxybenzaldehyde are 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 1.8 between mitomycin and 3-ethoxy-4-hydroxybenzaldehyde, *System suitability solution*

Tailing factor: NMT 1.3, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of mitomycin (C₁₅H₁₈N₄O₅) in the portion of Mitomycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Mitomycin RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of mitomycin in USP Mitomycin RS (µg/mg)

Acceptance criteria: NLT 970 µg/mg

IMPURITIES

• ORGANIC IMPURITIES

Solution A: 0.77 g/L of ammonium acetate

Solution B: Methanol and *Solution A* (20:80)

Solution C: Methanol and *Solution A* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
10	100	0
30	0	100
45	0	100
50	100	0

Standard solution: 0.025 mg/mL of USP Mitomycin RS in methanol

Sensitivity solution: 2.5 µg/mL of USP Mitomycin RS in methanol

Sample solution: 5 mg/mL of Mitomycin in methanol

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Sensitivity solution* and *Standard solution*

Suitability requirements

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Mitomycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of mitomycin from the *Standard solution*

C_S = concentration of USP Mitomycin RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of mitomycin in USP Mitomycin RS (µg/mg)

F_1 = conversion factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Albomitomycin C ^a	0.6	1.0	0.5
Mitomycin	1.0	—	—
Mitomycin B ^b	1.2	1.0	0.5
Cinnamamide	1.3	2.9	0.5
Mitomycin A ^c	1.6	1.0	0.5

^a [(1S,2S,4aS,8aR,9S,9aR)-7-Amino-9a-methoxy-6-methyl-5,8-dioxo-1,2,3,5,8,8a,9,9a-octahydro-1,2,4a-metheno-(epinitrilo)pyrrolo[1,2-a]indol-9-yl)methyl carbamate.

^b [(1aS,8S,8aR,8bS)-8a-Hydroxy-6-methoxy-1,5-dimethyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2',3':3,4]pyrrolo[1,2-a]indol-8-yl)methyl carbamate.

^c [(1aS,8S,8aR,8bS)-6,8a-Dimethoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2',3':3,4]pyrrolo[1,2-a]indol-8-yl)methyl carbamate.

Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	1.0	0.5
Total impurities	—	—	2.0

^a {(1S,2S,4aS,8aR,9S,9aR)-7-Amino-9a-methoxy-6-methyl-5,8-dioxo-1,2,3,5,8,8a,9,9a-octahydro-1,2,4a-metheno-(epinitrilo)pyrrolo[1,2-a]indol-9-yl)methyl carbamate.

^b {(1aS,8S,8aR,8bS)-8a-Hydroxy-6-methoxy-1,5-dimethyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2',3':3,4]pyrrolo[1,2-a]indol-8-yl)methyl carbamate.

^c {(1aS,8S,8aR,8bS)-6,8a-Dimethoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2',3':3,4]pyrrolo[1,2-a]indol-8-yl)methyl carbamate.

SPECIFIC TESTS

- **CRYSTALLINITY (695):** Meets the requirements

- **PH (791)**

Sample: 5-mg/mL suspension in water

Acceptance criteria: 6.0–7.5

- **WATER DETERMINATION, Method I (921):** NMT 2.5%
- **STERILITY TESTS (71):** Where the label states that Mitomycin is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Mitomycin is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 10.0 USP Endotoxin Units/mg of mitomycin.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS (11)**
USP Endotoxin RS
USP Mitomycin RS

Mitomycin for Injection**DEFINITION**

Mitomycin for Injection contains NLT 90.0% and NMT 120.0% of the labeled amount of mitomycin ($C_{15}H_{18}N_4O_5$).

IDENTIFICATION

- **A.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the Assay.

ASSAY• **PROCEDURE**

Mobile phase: Dissolve 1.54 g of ammonium acetate in 250 mL of methanol. Add 5.0 mL of 0.83 N acetic acid and water to make 1000 mL.

System suitability solution: 0.5 mg/mL of USP Mitomycin RS and 7.5 mg/mL of 3-ethoxy-4-hydroxybenzaldehyde in *N,N*-dimethylacetamide

Standard solution: 0.5 mg/mL of USP Mitomycin RS in *N,N*-dimethylacetamide

Sample solution: Add an accurately measured volume of *N,N*-dimethylacetamide to 1 container of Mitomycin for Injection to obtain a solution that is nominally 0.5 mg/mL of mitomycin.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 365 nm

Column: 3.9-mm × 30-cm; 10-μm packing L11

Flow rate: 2 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for mitomycin and 3-ethoxy-4-hydroxybenzaldehyde are 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 1.8 between mitomycin and 3-ethoxy-4-hydroxybenzaldehyde, *System suitability solution*

Tailing factor: NMT 1.3, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mitomycin ($C_{15}H_{18}N_4O_5$) in the container of Mitomycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Mitomycin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mitomycin in the *Sample solution* (mg/mL)

P = potency of mitomycin in USP Mitomycin RS (μg/mg)

F = conversion factor, 0.001 mg/μg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

SPECIFIC TESTS

- **PH (791)**

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 6.0–8.0 where it contains mannitol, and 5.5–8.5 where it contains hydroxypropyl betadex

- **WATER DETERMINATION, Method Ia (921)**

Sample solution: Prepare as directed for a hygroscopic specimen, using the pooled contents of five containers.

Acceptance criteria: NMT 5.0%

- **BACTERIAL ENDOTOXINS TEST (85):** Contains NMT 10.0 USP Endotoxin Units/mg of mitomycin
- **STERILITY TESTS (71):** Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)

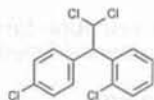
ADDITIONAL REQUIREMENTS**Change to read:**

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017), protected from light. Store at 25°, excursions permitted between 15° and 30°.

• **USP REFERENCE STANDARDS (11)**

USP Endotoxin RS
USP Mitomycin RS

Mitomane



$C_{14}H_{10}Cl_4$ 320.04
Benzene, 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]-, (RS)-;
(±)-1,1-Dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane [53-19-0].

DEFINITION

Mitomane contains NLT 97.0% and NMT 103.0% of mitotane ($C_{14}H_{10}Cl_4$), calculated on the anhydrous basis.
[CAUTION—Handle Mitotane with exceptional care, because it is a highly potent agent.]

IDENTIFICATION

- **A. INFRARED ABSORPTION (17M)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer: 1.38 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Buffer* (75:25)

System suitability solution: 0.2 mg/mL of USP

Mitomane RS and 0.2 mg/mL of the *p,p'*-isomer of mitotane in *Mobile phase*. [NOTE—The *p,p'*-isomer of mitotane is 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane known as *p,p'*-DDD.]

Standard solution: 0.2 mg/mL of USP Mitotane RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Mitotane in *Mobile phase*. [NOTE—Inject within 48 h of preparation.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for the *p,p'*-isomer of mitotane and mitotane are about 0.92 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between the *p,p'*-isomer of mitotane and mitotane, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mitotane ($C_{14}H_{10}Cl_4$) in the portion of Mitotane taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mitotane RS in the *Standard solution* (mg/mL)

C_U = concentration of Mitotane in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.5%

SPECIFIC TESTS

- **WATER DETERMINATION, Method Ia (921):** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**
USP Mitotane RS

Mitomane Tablets

» Mitotane Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mitotane ($C_{14}H_{10}Cl_4$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Mitotane RS

Identification—Triturate a quantity of finely powdered Tablets, equivalent to about 500 mg of mitotane, with 10 mL of water, filter on a sintered-glass filter funnel, and wash the residue with two 5-mL portions of water. Transfer the residue to a small beaker, add 4 mL of alcohol, heat to boiling, and filter immediately. Allow the filtrate to cool, filter the crystals of mitotane, wash once with 2 mL of alcohol, and dry in vacuum at 60° for 2 hours: the IR absorption spectrum of a mineral oil dispersion of the mitotane so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Mitotane RS.

Disintegration (701): 15 minutes, the use of disks being omitted.

Uniformity of dosage units (905): meet the requirements.

Assay—

Standard preparation—Dissolve about 50 mg of USP Mitotane RS, accurately weighed, in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution having a known concentration of about 200 μg per mL.

Assay preparation—Weigh and finely powder not less than 10 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of mitotane, to a 250-mL volumetric flask, add 100 mL of methanol, and shake occasionally for 5 minutes, then dilute with methanol to volume, and mix. Filter, rejecting the first portion of the filtrate, transfer 25.0 mL of the filtrate to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 268 nm, with a suitable spectrophotometer, using methanol as the blank.

Calculate the quantity, in mg, of $C_{14}H_{10}Cl_4$ in the portion of Tablets taken by the formula:

$$0.5C(A_U/A_S)$$

in which C is the concentration, in mg/mL, of USP Mitotane RS in the *Standard preparation*, and A_U and A_S are the ab-

sorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Mitoxantrone Injection

» Mitoxantrone Injection is a sterile solution of Mitoxantrone Hydrochloride in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 105.0 percent of the labeled amount of mitoxantrone ($C_{22}H_{28}N_4O_6$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

Labeling—Label Injection to state both the content of the active moiety and the name of the salt used in formulating the article. Label Mitoxantrone Injection to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration.

USP Reference standards (11)—

USP Mitoxantrone Hydrochloride RS

USP Mitoxantrone System Suitability Mixture RS

A mixture of 9,10-anthracenedione, 8-amino-1,4-dihydroxy-5-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-, hydrochloride ($C_{18}H_{19}N_3O_5 \cdot HCl$ 393.83) and USP Mitoxantrone Hydrochloride RS.

Identification—Transfer a volume of Injection, equivalent to about 2 mg of mitoxantrone, to a 200-mL volumetric flask, add 100 mL of water and 20 mL of 1 N hydrochloric acid, dilute with water to volume, and mix: the UV absorption spectrum of this solution exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Mitoxantrone Hydrochloride RS.

Bacterial Endotoxins Test (85)—It contains not more than 5 Endotoxin Units per mg of mitoxantrone.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, the entire contents of each container being used.

pH (791): between 3.0 and 4.5.

Chromatographic purity—Using the chromatogram of the *Assay preparation* obtained as directed in the *Assay*, calculate the percentage of each impurity in the Injection taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the response of any individual peak, other than the main mitoxantrone peak; and r_s is the sum of the responses of all the peaks in the chromatogram, including that of the main mitoxantrone peak: not more than 1.5% of any individual impurity and not more than 3.0% of the total impurities is found.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Sodium 1-heptanesulfonate solution, Mobile phase, System suitability solution, and Chromatographic system—Proceed as directed in the *Assay* under *Mitoxantrone Hydrochloride*.

Standard preparation—Transfer about 23 mg of USP Mitoxantrone Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 40 mL of *Mobile phase*, and dissolve by sonicating for about 5 minutes. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 0.4 mg of mitoxantrone ($C_{22}H_{28}N_4O_6$) per mL.

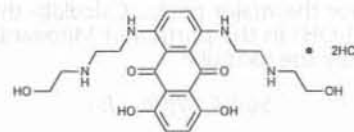
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 4 mg of mitoxantrone ($C_{22}H_{28}N_4O_6$), to a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Mitoxantrone Hydrochloride*. Calculate the quantity, in mg, of mitoxantrone ($C_{22}H_{28}N_4O_6$) in each mL of the Injection taken by the formula:

$$(444.49 / 517.40)(10C / V)(r_u / r_s)$$

in which 444.49 and 517.40 are the molecular weights of mitoxantrone and mitoxantrone hydrochloride, respectively; V is the volume, in mL, of the portion of Injection taken; and the other terms are as defined therein.

Mitoxantrone Hydrochloride



$C_{22}H_{28}N_4O_6 \cdot 2HCl$ 517.40

9,10-Anthracenedione, 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-, dihydrochloride. 1,4-Dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]anthraquinone dihydrochloride. [70476-82-3].

» Mitoxantrone Hydrochloride contains not less than 97.0 percent and not more than 102.0 percent of $C_{22}H_{28}N_4O_6 \cdot 2HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Mitoxantrone Hydrochloride RS

USP Mitoxantrone System Suitability Mixture RS

A mixture of 9,10-anthracenedione, 8-amino-1,4-dihydroxy-5-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-, hydrochloride ($C_{18}H_{19}N_3O_5 \cdot HCl$ 393.83) and USP Mitoxantrone Hydrochloride RS.

Identification, Infrared Absorption (197K).

Water Determination, Method I (921): not more than 6.0%.

Alcohol—

Standard solution—Transfer 5.0 mL of dehydrated alcohol to a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Internal standard solution—Transfer 5.0 mL of *n*-propyl alcohol to a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Standard preparation—Transfer 10.0 mL of the *Standard solution* to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, dilute with water to volume, and mix. This solution contains 0.063 mg of alcohol (C_2H_5OH) per mL.

Test preparation—Transfer about 100 mg of Mitoxantrone Hydrochloride, accurately weighed, to a 5-mL volumetric flask, add 2.0 mL of the *Internal standard solution*, dilute with water to volume, and mix. Sonicate for 2 minutes and shake for 2 minutes, repeating these actions until the specimen is completely dissolved.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 3-m column that contains 20% phase G1 and 0.1% phase G39 on silanized support S1A. Maintain the column at 50° for 5 minutes, then increase the temperature at a rate of 30° per minute. When 140° is reached, maintain that temperature for 20 minutes. Maintain the injection port at 200° and the detection block at 250°. Use helium as the carrier gas at a flow rate of about 15 mL per minute. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for alcohol and 1.0 for *n*-propyl alcohol, the resolution, *R*, between the alcohol and the *n*-propyl alcohol peaks is not less than 6.0, and the tailing factors for the two peaks are not more than 2.0.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 1 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of alcohol (C₂H₅OH) in the portion of Mitoxantrone Hydrochloride taken by the formula:

$$500(C/W)(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of alcohol (C₂H₅OH) in the *Standard preparation*; *W* is the weight, in mg, of Mitoxantrone Hydrochloride taken; and *R_U* and *R_S* are the ratios of the response of the alcohol peak to that of the *n*-propyl alcohol peak obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 1.5% is found.

Delete the following:

• **Heavy metals** (231)—Proceed as directed under *Method II*, except in the *Procedure* to filter the final solutions through a suitable acid-resistant membrane filter of 0.22 µm or finer porosity, instead of viewing them over a dark surface: the precipitate on the filter obtained from the *Test Preparation* is not darker than that obtained from the *Standard Preparation*. The limit is 0.002%. • (Official 1-Jan-2018)

Chromatographic purity—Using the chromatogram of the *Assay preparation* obtained as directed in the *Assay*, calculate the percentage of each impurity in the Mitoxantrone Hydrochloride taken by the formula:

$$100(r_i / r_s)$$

in which *r_i* is the response of any individual peak, other than the main mitoxantrone peak, and *r_s* is the sum of the responses of all the peaks in the chromatogram, including that of the main mitoxantrone peak: not more than 1.0% of any individual impurity and not more than 2.0% of total impurities is found.

Assay—

Sodium 1-heptanesulfonate solution—Dissolve 22.0 g of sodium 1-heptanesulfonate in about 150 mL of water, pass through a suitable filter having a 0.5-µm or finer porosity, and transfer the filtrate to a 250-mL volumetric flask. Wash the filter with about 50 mL of water, adding the filtrate to the 250-mL volumetric flask. Add 32.0 mL of glacial acetic acid to the volumetric flask, dilute with water to volume, and mix.

Mobile phase—Prepare a suitable degassed mixture of water, acetonitrile, and *Sodium 1-heptanesulfonate solution* (750:250:25). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability solution—Prepare a solution of USP Mitoxantrone System Suitability Mixture RS in a suitable volume of *Mobile phase* to obtain a solution containing about

0.2 mg of 8-amino-1,4-dihydroxy-5[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione hydrochloride (mitoxantrone related compound A) and 0.1 mg of mitoxantrone hydrochloride per mL.

Standard preparation—Transfer about 20 mg of USP Mitoxantrone Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 40 mL of *Mobile phase*, and dissolve by sonicating for about 5 minutes. Cool to room temperature, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer about 20 mg of Mitoxantrone Hydrochloride, accurately weighed, to a 50-mL volumetric flask, add 40 mL of *Mobile phase*, and dissolve by sonicating for about 5 minutes. Cool to room temperature, dilute with *Mobile phase* to volume, and mix.

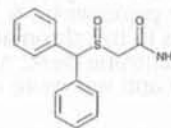
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The flow rate is about 3 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for mitoxantrone and 1.0 for mitoxantrone related compound A; the resolution, *R*, between mitoxantrone and mitoxantrone related compound A is not less than 3.0; and the tailing factor for the mitoxantrone peak is not more than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor, *k'*, for mitoxantrone is not less than 3.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. [NOTE—After use, wash the column with a mixture of acetonitrile and water (50:50), and store in this mixture.] Calculate the quantity, in mg, of C₂₂H₂₈N₄O₆ · 2HCl in the portion of Mitoxantrone Hydrochloride taken by the formula:

$$50C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of anhydrous mitoxantrone hydrochloride in the *Standard preparation*, as determined from the content of USP Mitoxantrone Hydrochloride RS corrected for the water content determined by a titrimetric water determination; and *r_U* and *r_S* are the mitoxantrone peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Modafinil



C₁₅H₁₅NO₂S

273.35

Acetamide, 2-[(diphenylmethyl)sulfinyl]-;
2-[(Diphenylmethyl)sulfinyl]-acetamide [68693-11-8].

DEFINITION

Modafinil contains NLT 98.0% and NMT 101.5% of C₁₅H₁₅NO₂S, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

ASSAY

- **PROCEDURE**

Buffer: 6.8 g/L of potassium dihydrogen phosphate in water. Adjust with phosphoric acid to a pH of 2.3.

Mobile phase: Acetonitrile and Buffer (35:65)
Diluent: Acetonitrile and water (35:65)
System suitability solution: 5 µg/mL of USP Modafinil RS and 10 µg/mL of USP Salicylic Acid RS in *Diluent*
Standard solution: 0.1 mg/mL of USP Modafinil RS in *Diluent*

Sample solution: 0.1 mg/mL of Modafinil in *Diluent*
Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for modafinil and salicylic acid are about 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.3 between modafinil and salicylic acid

Tailing factor: NMT 1.5 for the modafinil peak

Relative standard deviation: NMT 2.0% for the modafinil peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of modafinil ($C_{15}H_{15}NO_2S$) in the portion of Modafinil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Modafinil RS in the *Standard solution* (mg/mL)

C_U = concentration of Modafinil in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm (Official 1, Jan-2018)

ORGANIC IMPURITIES

Buffer, Mobile phase, Sample solution, System suitability solution, and Chromatographic system: Prepare as directed in the *Assay*.

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.3 between modafinil and salicylic acid

Tailing factor: NMT 1.5 for the modafinil peak

Relative standard deviation: NMT 2.0% for the modafinil peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Modafinil taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity

r_T = sum of the responses of all the peaks

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Modafinil	1.0	—	—
Salicylic acid ^a	1.1	—	—
Modafinil acid ^b	1.4	1.0	0.5
Modafinil sulfone ^c	1.7	0.9	0.5
Modafinil ester ^d	3.0	1.0	0.5
Any other individual unspecified impurity	—	1.0	0.05
Total impurities	—	—	1.0

^a Salicylic acid is used for calculating resolution and is not a potential impurity.

^b 2-[(Diphenylmethyl)sulfinyl]acetic acid.

^c 2-[(Diphenylmethyl)sulfonyl]acetamide.

^d 2-[(Diphenylmethyl)sulfinyl]acetic acid methyl ester.

SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* (921): NMT 0.2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

- **USP REFERENCE STANDARDS** (11)

USP Modafinil RS

USP Salicylic Acid RS

Modafinil Tablets

DEFINITION

Modafinil Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of modafinil ($C_{15}H_{15}NO_2S$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

Standard specimen: Transfer a quantity, in mg, of USP Modafinil RS, equivalent to the labeled amount of modafinil, to a suitable container. Add 50 mL each of dichloromethane and water. Shake the mixture, and allow the layers to separate. Filter a portion of the lower (dichloromethane) layer, and evaporate to dryness, using a stream of nitrogen if necessary. Prepare a potassium bromide pellet of the residue.

Sample specimen: Grind 1 Tablet, and add 50 mL each of dichloromethane and water. Shake the mixture, and allow the layers to separate. Filter a portion of the lower (dichloromethane) layer, and evaporate to dryness, using a stream of nitrogen if necessary. Prepare a potassium bromide pellet of the residue.

Acceptance criteria: Meet the requirements

ASSAY

- **PROCEDURE**

Buffer: 6.8 g/L of potassium dihydrogen phosphate in water. Adjust with phosphoric acid to a pH of 2.3.

Mobile phase: Acetonitrile and Buffer (35:65)

Diluent A: Acetonitrile and water (35:65)

Diluent B: Acetonitrile, water, and acetic acid (35:65:1)

System suitability solution: 5 µg/mL of USP Modafinil RS and 10 µg/mL of USP Salicylic Acid RS in *Diluent A*

Standard solution: 0.4 mg/mL of USP Modafinil RS in *Diluent B*

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 100 mg of modafinil, to a 250-mL volumetric flask, add 200 mL of *Diluent B*, and sonicate for about 5 min with intermittent manual shaking. Dilute with *Diluent B* to

volume, and mix. Pass through a suitable filter of 0.45- μ m or finer pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 5 μ L

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for modafinil and salicylic acid are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.3 between modafinil and salicylic acid

Tailing factor: NMT 1.5 for the modafinil peak

Relative standard deviation: NMT 2.0% for the modafinil peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of modafinil ($C_{15}H_{15}NO_2S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Modafinil RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of modafinil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: Prepare a solution having a known concentration of USP Modafinil RS in *Medium*.

Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium* if necessary

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: Absorption maximum at about 222 nm

Analysis

Samples: *Standard solution* and *Sample solution*
Determine the amount of modafinil ($C_{15}H_{15}NO_2S$) dissolved.

Tolerances: NLT 75% (Q) of the labeled amount of modafinil ($C_{15}H_{15}NO_2S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution* Test 2.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: ($L/900$) mg/mL of USP Modafinil RS, where L is the label claim (mg/Tablet). Prepare by dissolving the standard in a volume of methanol equivalent to 5%–10% of the final volume and then diluting with *Medium* to volume.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: Absorption maximum at about 225 nm

Cell: 0.1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of modafinil ($C_{15}H_{15}NO_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of modafinil ($C_{15}H_{15}NO_2S$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution* Test 3.

Medium: 0.1 N hydrochloric acid; 900 mL, deaerated

Apparatus 2: 75 rpm

Time: 30 min

Standard solution: ($L/900$) mg/mL of USP Modafinil RS, where L is the label claim (mg/Tablet). Prepare by dissolving the standard in a volume of methanol equivalent to 5%–10% of the final volume and then diluting with *Medium* to volume.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: Absorption maximum at about 220 nm

Cell: 0.1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of modafinil ($C_{15}H_{15}NO_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of modafinil ($C_{15}H_{15}NO_2S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Buffer, Mobile phase, *System suitability solution*, *Sample solution*, and *Chromatographic system*: Proceed as directed in the *Assay*.

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.3 between modafinil and salicylic acid

Relative standard deviation: NMT 2.0% for the modafinil peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity

r_T = sum of the responses of all the peaks

F = relative response factor (see Table 1)
Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Modafinil	1.0	—	—
Salicylic acid ^a	1.1	—	—
Modafinil acid ^b	1.4	1.0	0.5
Modafinil sulfone ^c	1.7	0.90	0.5
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	1.5

^a Salicylic acid is used for calculating resolution and is not a potential impurity.

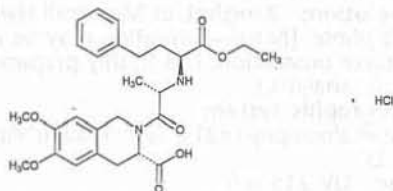
^b 2-[(Diphenylmethyl)sulfinyl]acetic acid.

^c 2-[(Diphenylmethyl)sulfonyl]acetamide.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
USP Modafinil RS
USP Salicylic Acid RS

Moexipril Hydrochloride



$C_{27}H_{34}N_2O_7 \cdot HCl$ 535.03
3-Isoquinolinecarboxylic acid, 2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-, monohydrochloride, [3S-[2[R*(R*)],3R*]]-; (3S)-2-[(2S)-N-[(1S)-1-Carboxy-3-phenylpropyl]alanyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid, 2-ethyl ester, monohydrochloride [82586-52-5].

DEFINITION

Moexipril Hydrochloride contains NLT 98.0% and NMT 102.0% of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (17K)**
- **B.** The relative retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL (191), Chloride:** Meets the requirements

ASSAY

PROCEDURE

Buffer: 1.32 g/L of dibasic ammonium phosphate. Adjust with diluted phosphoric acid to a pH of 7.5.

Solution A: Acetonitrile and tetrahydrofuran (95:5)

Mobile phase: *Solution A* and *Buffer* (30:70)

Standard solution: 0.1 mg/mL of USP Moexipril Hydrochloride RS in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution.]

Sample solution: 0.1 mg/mL of Moexipril Hydrochloride in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 10 μL

Run time: NLT 3.2 times the retention time of moexipril

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) in the portion of Moexipril Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Moexipril Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Moexipril Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 10 ppm (Official 1-Jan-2018)
- **RESIDUE ON IGNITION (281):** NMT 0.20%

Change to read:

ORGANIC IMPURITIES

[NOTE—Use freshly prepared samples for analysis.]

Solution A and Chromatographic system: Proceed as directed in the *Assay*.

Solution B: Proceed as directed for the *Buffer* in the *Assay*.

Diluent: *Solution A* and *Solution B* (20:80)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	20	80
5	20	80
35	55	45
65	55	45
70	20	80
80	20	80

Standard solution 1: 4 μg/mL of USP Moexipril Hydrochloride RS in *Diluent*. [NOTE—Sonication may be necessary for complete dissolution.]

Standard solution 2: 2 mg/mL of USP Moexipril Hydrochloride RS and 3 μg/mL each of USP Moexipril Related Compound A RS, USP Moexipril Related Compound B RS, USP Moexipril Related Compound C RS, USP Moexipril Related Compound D RS, USP Moexipril Related Compound E RS, USP Moexipril Related Compound F

RS, and USP Moexipril Related Compound G RS in *Diluent*. [NOTE—Sonication may be necessary for complete dissolution.]

Sample solution: 2 mg/mL of Moexipril Hydrochloride in *Diluent*. [NOTE—Sonication may be necessary for complete dissolution.]

System suitability

Samples: *Standard solution 1* and *Standard solution 2*

Suitability requirements

Resolution: NLT 3.5 between moexipril related compound A and moexipril related compound E; NLT 2.5 between moexipril and moexipril related compound G, *Standard solution 2*

Relative standard deviation: NMT 5.0% for moexipril, *Standard solution 1*

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Moexipril Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of each specified impurity from the *Sample solution*

r_s = peak response of the corresponding Reference Standard from *Standard solution 2*

C_s = concentration of each specified impurity in *Standard solution 2* (mg/mL)

C_u = concentration of Moexipril Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity in the portion of Moexipril Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of each individual unspecified impurity from the *Sample solution*

r_s = peak response of moexipril from *Standard solution 1*

C_s = concentration of USP Moexipril Hydrochloride RS in *Standard solution 1* (mg/mL)

C_u = concentration of Moexipril Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard peaks less than 0.05%. (RB 1-Jun-2016)

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Moexipril related compound E ^a	0.14	0.15
Moexipril related compound A ^b	0.28	0.2
Moexipril related compound F ^c	0.62	0.15
Moexipril related compound G ^d	0.90	0.15

^a (S)-6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

^b (3S)-2-[(2S)-N-[(1S)-1-Carboxy-3-phenylpropyl]alaninyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid.

^c (S)-2-[(S)-1-Ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoic acid.

^d (S)-6,7-Dimethoxy-2-[(S)-2-[(S)-1-methoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

^e (S)-2-[(S)-2-[(S)-4-Cyclohexyl-1-ethoxy-1-oxobutan-2-ylamino]propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

^f (S)-Ethyl 2-[(3S,11aS)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1H-pyrazino[1,2-b]isoquinolin-2(6H,11H,11aH)-yl]-4-phenylbutanoate.

^g (S)-tert-Butyl 2-[(S)-2-[(S)-1-ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate.

^h Sum of all specified and unspecified impurities.

Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Moexipril	1.00	—
Moexipril related compound D ^e	1.28	0.15
Moexipril related compound B ^f	1.62	0.2
Moexipril related compound C ^g	2.26	0.15
Any other individual unspecified impurity	—	0.10
Total impurities ^h	—	1.0% (RB 1-Jun-2016)

^a (S)-6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

^b (3S)-2-[(2S)-N-[(1S)-1-Carboxy-3-phenylpropyl]alaninyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid.

^c (S)-2-[(S)-1-Ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoic acid.

^d (S)-6,7-Dimethoxy-2-[(S)-2-[(S)-1-methoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

^e (S)-2-[(S)-2-[(S)-4-Cyclohexyl-1-ethoxy-1-oxobutan-2-ylamino]propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

^f (S)-Ethyl 2-[(3S,11aS)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1H-pyrazino[1,2-b]isoquinolin-2(6H,11H,11aH)-yl]-4-phenylbutanoate.

^g (S)-tert-Butyl 2-[(S)-2-[(S)-1-ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate.

^h Sum of all specified and unspecified impurities.

• CONTENT OF IMIDAZOLE

Mobile phase: Hexane, isopropyl alcohol, and diethylamine (52:48:0.025)

Standard solution: 0.01 mg/mL of USP Imidazole RS in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution.]

Sample solution: 2 mg/mL of Moexipril Hydrochloride in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution. Use freshly prepared *Sample solution* for analysis.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-μm packing L3

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: NLT 3.3 times the retention time of imidazole

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of imidazole in the portion of Moexipril Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of imidazole from the *Sample solution*

r_s = peak response of imidazole from the *Standard solution*

C_s = concentration of USP Imidazole RS in the *Standard solution* (mg/mL)

C_u = concentration of Moexipril Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.03%

SPECIFIC TESTS

• **WATER DETERMINATION** (921), *Method I*, *Method Ia*: NMT 1.5%

• **OPTICAL ROTATION** (781S), *Procedures*, *Specific Rotation*
Sample solution: 0.011 g/mL of Moexipril Hydrochloride in alcohol. Sonicate to dissolve the sample.

Acceptance criteria: +30.0° to +38.0°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from moisture. Store at room temperature.
- **USP REFERENCE STANDARDS (11)**
 - USP Imidazole RS
 - USP Moexipril Hydrochloride RS
 - USP Moexipril Related Compound A RS
(3*S*)-2-[(2*S*)-*N*-[(1*S*)-1-Carboxy-3-phenylpropyl]alanyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinoline-carboxylic acid.
 $C_{25}H_{30}N_2O_7$ 470.51
 - USP Moexipril Related Compound B RS
(*S*)-Ethyl 2-[(3*S*,11*aS*)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1*H*-pyrazino[1,2-*b*]isoquinolin-2(6*H*,11*H*,11*aH*)-yl]-4-phenylbutanoate.
 $C_{27}H_{32}N_2O_6$ 480.55
 - USP Moexipril Related Compound C RS
(*S*)-*tert*-Butyl 2-[(*S*)-2-[(*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate.
 $C_{31}H_{42}N_2O_7$ 554.67
 - USP Moexipril Related Compound D RS
(*S*)-2-[(*S*)-2-[(*S*)-4-Cyclohexyl-1-ethoxy-1-oxobutan-2-ylamino]propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
 $C_{27}H_{40}N_2O_7$ 504.62
 - USP Moexipril Related Compound E RS
(*S*)-6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
 $C_{12}H_{15}NO_4$ 237.25
 - USP Moexipril Related Compound F RS
(*S*)-2-[(*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoic acid.
 $C_{15}H_{21}NO_4$ 279.33
 - USP Moexipril Related Compound G RS
(*S*)-6,7-Dimethoxy-2-[(*S*)-2-[(*S*)-1-methoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
 $C_{26}H_{32}N_2O_7$ 484.54

Moexipril Hydrochloride Tablets

DEFINITION

Moexipril Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$).

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION (197U)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Buffer: 0.01 M potassium dihydrogen phosphate
Diluent: Acetonitrile and water (30:70)
Mobile phase: Acetonitrile and *Buffer* (350:650)
Standard solution: 0.075 mg/mL of USP Moexipril Hydrochloride RS in *Diluent*. Initially fill with *Diluent* to about 70% of the total volume, and sonicate. Further dilute with *Diluent* to volume.
Sample solution: Nominally 0.075 mg/mL of moexipril hydrochloride in *Diluent*, prepared from a sufficient number of crushed Tablets as follows. Add *Diluent* to about 75% of the total volume, and sonicate for 30 min with intermittent shaking. Dilute with *Diluent* to volume, and pass through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 45°

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

Run time: 4 times the retention time of the moexipril peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Moexipril Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of moexipril hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Test 1

Buffer, Diluent, Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 15 min

Standard stock solution: 0.16 mg/mL of USP Moexipril Hydrochloride RS in *Diluent*. [NOTE—Sonication may be necessary for complete dissolution.]

Standard solution: 0.016 mg/mL of USP Moexipril Hydrochloride RS in *Medium* from the *Standard stock solution* for 15-mg Tablet strength and 0.008 mg/mL of USP Moexipril Hydrochloride RS in *Medium* from the *Standard stock solution* for 7.5-mg Tablet strength

Sample solution: Pass 10 mL of the solution under test through a suitable filter of 0.45- μ m pore size, discarding the first 2–3 mL.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of moexipril from the *Sample solution*

r_S = peak response of moexipril from the *Standard solution*

C_S = concentration of USP Moexipril Hydrochloride RS in the *Standard solution*

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Buffer, Diluent, Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Medium, Apparatus 2, Standard stock solution, Standard solution, Sample solution, and Analysis: Proceed as directed in Test 1.

Time: 30 min

Tolerances: NLT 80% (Q) of the labeled amount of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements for Content Uniformity

IMPURITIES

Change to read:

• ORGANIC IMPURITIES

Solution A: 0.025% trifluoroacetic acid in water

Solution B: Acetonitrile and tetrahydrofuran (90:10)

Diluent: Acetonitrile and water (30:70)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
50	30	70
60	95	5
70	95	5

Impurity stock solution: 0.12 mg/mL of USP Moexipril Related Compound G RS in *Diluent*. [NOTE—Sonication may be necessary for complete dissolution.]

System suitability solution: 1.2 mg/mL of USP Moexipril Hydrochloride RS and 2.4 µg/mL of USP Moexipril Related Compound G RS from the *Impurity stock solution* in *Diluent*. [NOTE—Sonication may be necessary for complete dissolution.]

Standard stock solution: 1.2 mg/mL of USP Moexipril Hydrochloride RS in *Diluent*. Initially add *Diluent* to about 60% of the volume of the flask, and sonicate with intermittent shaking for complete dissolution.

Standard solution: 6 µg/mL each of USP Moexipril Related Compound A RS and USP Moexipril Related Compound B RS, and 1.2 µg/mL of USP Moexipril Hydrochloride RS in *Diluent* from the *Standard stock solution*. [NOTE—Sonication may be necessary for complete dissolution.]

Sample solution: Nominally 1.2 mg/mL of moexipril hydrochloride in *Diluent*, prepared from a sufficient number of crushed Tablets. Initially add *Diluent* to about 60% of the volume of the flask, and sonicate for 20 min with intermittent shaking in ice cold water. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.5 between moexipril and moexipril related compound G, *System suitability solution*

Tailing factor: NMT 2.0 for the moexipril peak, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *System suitability solution*, *Standard solution*, and *Sample solution*

Calculate the percentage of moexipril related compound A and moexipril related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of moexipril related compound A and moexipril related compound B from the *Sample solution*

r_S = peak response of moexipril related compound A and moexipril related compound B from the *Standard solution*

C_S = concentration of USP Moexipril Related Compound A RS and USP Moexipril Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of moexipril hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any other individual unspecified degradation product from the *Sample solution*

r_S = peak response of moexipril from the *Standard solution*

C_S = concentration of USP Moexipril Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of moexipril hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard peaks less than 0.1%. (RB 1-Jun-2016)

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Moexipril related compound E ^a	0.31	—
Moexipril related compound F ^a	0.77	—
Moexipril related compound A ^b	0.85	2.0
Moexipril related compound G ^a	0.94	—
Moexipril	1.00	—
Moexipril related compound D ^a	1.17	—
Moexipril related compound C ^a	1.27	—
Moexipril related compound B ^c	1.43	1.5
Any unspecified degradation product	—	0.2% (RB 1-Jun-2016)
Total impurities ^d	—	2.0

^a Process-related impurities controlled in the drug substance.

^b (3S)-2-[(2S)-N-[(1S)-1-Carboxy-3-phenylpropyl]alanyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid.

^c (S)-Ethyl 2-[(3S,11aS)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1H-pyrazino[1,2-b]isoquinolin-2(6H,11H,11aH)-yl]-4-phenylbutanoate.

^d Total impurities do not include moexipril related compound A.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store at controlled room temperature in tight, well-closed containers, and protect from moisture.

- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
 - USP Moexipril Hydrochloride RS
 - USP Moexipril Related Compound A RS
(3*S*)-2-[(2*S*)-*N*-[(1*S*)-1-Carboxy-3-phenylpropyl]alan-yl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinoline-carboxylic acid.
 $C_{25}H_{30}N_2O_7$ 470.51
 - USP Moexipril Related Compound B RS
(*S*)-Ethyl 2-[(3*S*,11*aS*)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1*H*-pyrazino[1,2-*b*]isoquinolin-2(6*H*,11*H*,11*aH*)-yl]-4-phenylbutanoate.
 $C_{27}H_{32}N_2O_6$ 480.55
 - USP Moexipril Related Compound G RS
(*S*)-6,7-Dimethoxy-2-[(*S*)-2-[(*S*)-1-methoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.
 $C_{26}H_{32}N_2O_7$ 484.54

Moexipril Hydrochloride and Hydrochlorothiazide Tablets

DEFINITION

Moexipril Hydrochloride and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% each of the labeled amounts of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer: 0.01 M potassium dihydrogen phosphate

Mobile phase: Acetonitrile and *Buffer* (350:650)

Diluent: Acetonitrile and water (30:70)

Standard solution: Prepare solutions of USP Moexipril Hydrochloride RS and USP Hydrochlorothiazide RS in *Diluent*, of concentrations stated in *Table 1*. Initially add *Diluent* to 70% of the total volume, sonicate to dissolve, and then dilute with *Diluent* to volume.

Table 1

Tablet Strength Moexipril Hydrochloride/ Hydrochlorothiazide (mg/mg)	Concentration of Moexipril Hydrochloride (mg/mL)	Concentration of Hydrochlorothiazide (mg/mL)
7.5/12.5	0.06	0.1
15/12.5	0.06	0.05
15/25	0.06	0.1

Sample solution: Prepare the *Sample solutions* of nominal concentrations given in *Table 1* from NLT 20 powdered Tablets as follows. Initially add *Diluent* to about 60% of the total volume, sonicate for 45 min with intermittent shaking, and then dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: 2.2 times the retention time of the moexipril peak

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2500 theoretical plates for moexipril and NLT 4000 theoretical plates for the hydrochlorothiazide peaks

Tailing factor: NMT 2.0 for both moexipril and hydrochlorothiazide peaks

Relative standard deviation: NMT 2.0% for both moexipril and hydrochlorothiazide peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of moexipril or hydrochlorothiazide from the *Sample solution*

r_S = peak response of moexipril or hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Moexipril Hydrochloride RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of moexipril hydrochloride or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of the labeled amounts of moexipril hydrochloride and hydrochlorothiazide

PERFORMANCE TESTS

• DISSOLUTION (711)

Buffer, Mobile phase, Diluent, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 15 min

Standard solution: Prepare solutions of USP Moexipril Hydrochloride RS and USP Hydrochlorothiazide RS in *Medium* of concentrations stated in *Table 2*.

Table 2

Tablet Strength Moexipril Hydrochloride/ Hydrochlorothiazide (mg/mg)	Concentration of USP Moexipril Hydrochloride RS (μ g/mL)	Concentration of USP Hydrochlorothiazide RS (μ g/mL)
7.5/12.5	8	14
15/12.5	16	14
15/25	16	28

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size, discarding the first 2–3 mL.

Analysis**Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amounts of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of moexipril or hydrochlorothiazide from the *Sample solution*

r_S = peak response of moexipril or hydrochlorothiazide from the *Standard solution*

C_S = concentration of USP Moexipril Hydrochloride RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

L = label claim for moexipril hydrochloride or hydrochlorothiazide (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 70% (Q) of the labeled amounts each of moexipril hydrochloride ($C_{27}H_{34}N_2O_7 \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) are dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

IMPURITIES• **ORGANIC IMPURITIES**

Solution A: Add 1 mL of trifluoroacetic acid to 4 L of water.

Solution B: Acetonitrile and tetrahydrofuran (90:10)

Mobile phase: See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	95	5
50	30	70
60	95	5
70	95	5

Diluent: Proceed as directed in the *Assay*.

System suitability solution: 1.2 mg/mL of USP Moexipril Hydrochloride RS, 2 mg/mL of USP Hydrochlorothiazide RS, and 2.4 µg/mL of USP Moexipril Related Compound G RS in *Diluent*. Initially add *Diluent* to 70% of the total volume, sonicate to dissolve, and then dilute with *Diluent* to volume.

Standard solution: 1.2 µg/mL of USP Moexipril Hydrochloride RS, 12 µg/mL each of USP Moexipril Related Compound A RS and USP Moexipril Related Compound B RS, 2 µg/mL of USP Hydrochlorothiazide RS, and 40 µg/mL each of USP Benzothiadiazine Related Compound A RS and USP Chlorothiazide RS in *Diluent*. Initially add *Diluent* to 70% of the total volume, sonicate to dissolve, and then dilute with *Diluent* to volume.

Sample solution: Prepare solutions of nominal concentration given in Table 4. Initially add *Diluent* to 70% of the total volume, and sonicate for 15 min with intermittent shaking in ice cold water. Dilute with *Diluent* to volume, and pass through a suitable filter of 0.45-µm pore size.

Table 4

Tablet Strength Moexipril Hydrochloride/ Hydrochlorothiazide (mg/mg)	Number of Tablets (NLT)	Nominal Concentration of Moexipril Hydrochloride (mg/mL)	Nominal Concentration of Hydrochlorothiazide (mg/mL)
7.5/12.5	20	1.2	2
15/12.5	10	1.8	1.5
15/25	10	1.2	2

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.5 between the moexipril and moexipril related compound G peaks, *System suitability solution*

Tailing factor: NMT 2.0 for both moexipril and hydrochlorothiazide peaks, *Standard solution*

Relative standard deviation: NMT 5.0% for both moexipril and hydrochlorothiazide peaks, *Standard solution*

Analysis**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of moexipril related compound A and moexipril related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of moexipril related compound A or moexipril related compound B from the *Sample solution*

r_S = peak response of USP Moexipril Related Compound A RS or USP Moexipril Related Compound B RS from the *Standard solution*

C_S = concentration of USP Moexipril Related Compound A RS and USP Moexipril Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of moexipril hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of benzothiadiazine related compound A or chlorothiazide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzothiadiazine related compound A or chlorothiazide from the *Sample solution*

r_S = peak response of benzothiadiazine related compound A or chlorothiazide from the *Standard solution*

C_S = concentration of USP Benzothiadiazine Related Compound A RS or USP Chlorothiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- r_u = peak response of any other individual impurity from the *Sample solution*
 r_s = peak response of moexipril from the *Standard solution*
 C_s = concentration of USP Moexipril Hydrochloride RS in the *Standard solution* (mg/mL)
 C_u = nominal concentration of moexipril hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 5.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Moexipril related compound E ^{a,b}	0.31	—
Benzothiadiazine related compound A ^c	0.47	1.0
Chlorothiazide ^d	0.53	0.5
Hydrochlorothiazide	0.57	—
5-Chlorohydrochlorothiazide ^{e,b}	0.82	—
Moexipril related compound F ^b	0.77	—
Moexipril related compound A ^g	0.85	1.0
Moexipril related compound G ^{h,b}	0.94	—
Moexipril	1.00	—
Moexipril related compound D ^{i,b}	1.17	—
Moexipril related compound C ^{i,b}	1.27	—
Moexipril related compound B ^k	1.43	1.5
Any other individual unspecified impurity	—	0.2
Total impurities	—	4.0 ^l

^a (S)-6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

^b Process-related impurity controlled in the drug substance.

^c 4-Amino-6-chloro-1,3-benzenedisulfonamide.

^d 2H-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-, 1,1-dioxide.

^e 5-Chloro-2H-1,2,4-benzothiadiazine-7-sulfonamide, 6-chloro-3,4-dihydro-, 1,1-dioxide.

^f (S)-2-[(S)-1-Ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoic acid.

^g (3S)-2-[(2S)-N-[(1S)-1-Carboxy-3-phenylpropyl]alanyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid.

^h (S)-6,7-Dimethoxy-2-[(S)-2-[(S)-1-methoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

ⁱ (S)-2-[(S)-2-[(S)-4-Cyclohexyl-1-ethoxy-1-oxobutan-2-ylamino]propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

^j (S)-tert-Butyl 2-[(S)-2-[(S)-1-ethoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate.

^k (S)-Ethyl 2-[(3S,11aS)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1H-pyrazino[1,2-b]isoquinolin-2(6H,11H,11aH)-yl]-4-phenylbutanoate.

^l Total impurities is a sum total of all specified and unspecified impurities.

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers, and protect from light. Store at controlled room temperature.

USP REFERENCE STANDARDS (11)

USP Benzothiadiazine Related Compound A RS

4-Amino-6-chloro-1,3-benzenedisulfonamide.

C₆H₈ClN₃O₄S₂ 285.73

USP Chlorothiazide RS

2H-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-, 1,1-dioxide.

C₇H₆ClN₃O₄S₂ 295.73

USP Hydrochlorothiazide RS

USP Moexipril Hydrochloride RS

USP Moexipril Related Compound A RS

(3S)-2-[(2S)-N-[(1S)-1-Carboxy-3-phenylpropyl]alanyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid.

C₂₅H₃₀N₂O₇ 470.51

USP Moexipril Related Compound B RS

(S)-Ethyl 2-[(3S,11aS)-8,9-dimethoxy-3-methyl-1,4-dioxo-3,4-dihydro-1H-pyrazino[1,2-b]isoquinolin-2(6H,11H,11aH)-yl]-4-phenylbutanoate.

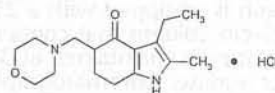
C₂₇H₃₂N₂O₆ 480.55

USP Moexipril Related Compound G RS

(S)-6,7-Dimethoxy-2-[(S)-2-[(S)-1-methoxy-1-oxo-4-phenylbutan-2-ylamino]propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

C₂₆H₃₂N₂O₇ 484.54

Molindone Hydrochloride



C₁₆H₂₄N₂O₂ · HCl 312.83

4H-Indol-4-one, 3-ethyl-1,5,6,7-tetrahydro-2-methyl-5-(4-morpholinylmethyl)-, monohydrochloride.

3-Ethyl-6,7-dihydro-2-methyl-5-(morpholinomethyl)indol-4(5H)-one monohydrochloride [15622-65-8].

» Molindone Hydrochloride contains not less than 98.0 percent and not more than 101.5 percent of C₁₆H₂₄N₂O₂ · HCl, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Molindone Hydrochloride RS

Identification—

A: Infrared Absorption (197K). Do not dry specimens.

B: Prepare a solution in methanol containing 10 mg of molindone hydrochloride per mL. Separately apply 1 µL of this solution and 1 µL of a Standard solution containing 10 mg per mL of USP Molindone Hydrochloride RS in methanol to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow the spots to dry. Protect the chromatogram from light, and develop in a solvent system consisting of a mixture of alcohol, methanol, and 1 N hydrochloric acid (90:5:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a freshly prepared solution containing 100 mg of potassium ferricyanide dissolved in 20 mL of 10% ferric chloride solution: the principal spot obtained from the test solution corresponds in R_F value and intensity to that obtained from the Standard solution.

C: It responds to the tests for *Chloride* (191).

pH (791): between 4.0 and 5.0, in a solution (1 in 100).

Water Determination, Method I (921): not more than 0.5%.

Residue on ignition (281): not more than 0.25%.

Delete the following:

• **Heavy metals, Method II** (231): not more than 0.003%.

• (Official 1-Jan-2018)

Chromatographic purity—

Mobile phase—Dissolve 1.1 g of sodium octanesulfonate in 600 mL of water, add 400 mL of methanol, 1 mL of glacial acetic acid, and 0.5 mL of triethylamine. Mix, filter through a filter having a porosity of 0.45 μ m or less, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Solvent mixture—Proceed as directed in the Assay.

Standard solution—Prepare a solution of USP Molindone Hydrochloride RS in *Solvent mixture* having a known concentration of about 0.01 mg per mL.

Test solution—Transfer about 100 mg of Molindone Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Solvent mixture* to volume.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm \times 25-cm column that contains packing L11. The column temperature is maintained at 35°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses of all peaks: no peak from the *Test solution*, other than the molindone peak, is greater than the molindone peak from the *Standard preparation* (0.5%), and the sum of all the impurity peaks is not greater than 2.0%.

Assay—

Mobile phase—Dissolve 1.1 g of sodium octanesulfonate in 480 mL of water, add 520 mL of methanol, 2 mL of glacial acetic acid, and 0.4 mL of triethylamine. Mix, filter through a 0.45- μ m filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Solvent mixture—Prepare a mixture of 0.01 N hydrochloric acid and methanol (60:40).

Internal standard solution—Dissolve 200 mg of butylparaben in 40 mL of methanol in a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard preparation—Transfer about 25 mg of USP Molindone Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix.

Assay preparation—Transfer about 50 mg of Molindone Hydrochloride, accurately weighed, to a 100-mL volumetric flask. Add 10.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm \times 25-cm column that contains packing L11. The column temperature is maintained at 35°. The flow rate is 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the resolution, R , between the molindone and butylparaben peaks is not less than 2, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for molindone and 1.0 for

butylparaben. Calculate the quantity, in mg, of $C_{16}H_{24}N_2O_2 \cdot HCl$ in the portion of Molindone Hydrochloride taken by the formula:

$$100C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Molindone Hydrochloride RS in the *Standard preparation*, and R_U and R_S are the ratios of the peak response of molindone to that of butylparaben obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Molindone Hydrochloride Tablets

» Molindone Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of molindone hydrochloride ($C_{16}H_{24}N_2O_2 \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Molindone Hydrochloride RS

Identification—Dissolve a portion of finely powdered Tablets in methanol to obtain a test solution containing about 2.5 mg of molindone hydrochloride per mL. Separately apply 5 μ L of the test solution and 5 μ L of a *Standard solution* of USP Molindone Hydrochloride RS in methanol containing 2.5 mg per mL to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, protect the chromatogram from light, and develop in a solvent system consisting of a mixture of alcohol and 1 N hydrochloric acid (95:5). Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by spraying with Dragendorff's reagent, prepared as directed for *Visualization Technique 3* under *Ordinary Impurities* (466): the R_f value of the principal spot obtained from the test solution corresponds to that obtained from the *Standard solution*.

Uniformity of dosage units (905): meet the requirements.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Solvent A—Mix 300 mL of methanol and 700 mL of 0.1 N hydrochloric acid.

Solvent B—Mix 75 mL of methanol and 25 mL of 0.1 N hydrochloric acid.

Standard solution—Transfer about 100 mg of USP Molindone Hydrochloride RS, accurately weighed, to a 250-mL volumetric flask, and dissolve in and dilute with *Solvent A* to volume. Pipet 5.0 mL of this stock solution into a 250-mL volumetric flask, and dilute with *Solvent A* to volume. Pipet 15.0 mL of the diluted stock solution into a 50-mL volumetric flask, and dilute with *Solvent A* to volume.

Test solution—Withdraw a portion of the solution under test, and filter, discarding the first 3 mL of filtrate. Pipet 15.0 mL of this solution into a 25-mL volumetric flask, and dilute with *Solvent B* to volume.

Mobile phase—Dissolve 1.08 g of sodium 1-octanesulfonate in 480 mL of water. Add 520 mL of methanol, 2.0 mL of acetic acid, and 0.4 mL of triethylamine, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm UV detec-

tor and a 4.6-mm × 25-cm column that contains packing L11. The flow rate is about 1.5 mL per minute.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak heights. Determine the amount of molindone hydrochloride ($C_{16}H_{24}N_2O_2 \cdot HCl$) dissolved.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{16}H_{24}N_2O_2 \cdot HCl$ is dissolved in 30 minutes.

Assay—

Mobile phase, Solvent mixture, Internal standard solution, Standard preparation, and Chromatographic system—Proceed as directed in the Assay under Molindone Hydrochloride.

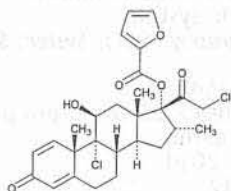
Assay preparation—Accurately weigh not less than 20 Tablets, grind the Tablets to a homogeneous mixture, and transfer an accurately weighed portion, equivalent to about 50 mg of molindone hydrochloride, to a 250-mL conical flask. Add 10.0 mL of *Internal standard solution* and 90.0 mL of *Solvent mixture*, shake for 30 minutes, and filter.

Procedure—Proceed as directed for *Procedure* in the Assay under Molindone Hydrochloride. Calculate the quantity, in mg, of molindone hydrochloride ($C_{16}H_{24}N_2O_2 \cdot HCl$) in the portion of Tablets taken by the formula:

$$100C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Molindone Hydrochloride RS in the *Standard preparation*, and R_U and R_S are the ratios of the peak response of molindone to that of butylparaben obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Mometasone Furoate



$C_{27}H_{30}Cl_2O_6$ 521.43
Pregna-1,4-diene-3,20-dione, 9,21-dichloro-17-[(2-furanylcarbonyl)oxy]-11-hydroxy-16-methyl-, (11β,16α)-; 9,21-Dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate) [83919-23-7].

DEFINITION

Mometasone Furoate contains NLT 97.0% and NMT 102.0% of mometasone furoate ($C_{27}H_{30}Cl_2O_6$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the Assay.

ASSAY

PROCEDURE

Mobile phase: Methanol and water (65:35)

Diluent: Methanol, acetic acid, and water (65:0.2:35)

Internal standard solution: 0.4 mg/mL of

beclomethasone dipropionate in *Diluent*

Standard stock solution: 0.1 mg/mL of USP

Mometasone Furoate RS, prepared by dissolving USP

Mometasone Furoate RS in methanol and diluting

quantitatively and stepwise, if necessary, with *Diluent*

Standard solution: 0.02 mg/mL of USP Mometasone Furoate RS and 0.08 mg/mL of beclomethasone dipropionate, prepared by pipetting equal volumes of *Standard stock solution* and *Internal standard solution* into a suitable volumetric flask and diluting with *Diluent* to volume, if necessary

Sample stock solution: 0.1 mg/mL of mometasone furoate, prepared by dissolving Mometasone Furoate in methanol and diluting quantitatively and stepwise, if necessary, with *Diluent*

Sample solution: 0.02 mg/mL of mometasone furoate and 0.08 mg/mL of beclomethasone dipropionate, prepared by pipetting 10 mL each of *Sample stock solution* and *Internal standard solution* into a 50-mL volumetric flask and diluting with *Diluent* to volume

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 1.7 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for mometasone furoate and beclomethasone dipropionate are about 1.0 and 1.6, respectively.]

Suitability requirements

Resolution: NLT 4.0 between the mometasone furoate and beclomethasone dipropionate peaks

Tailing factor: NMT 1.8 for the mometasone furoate peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mometasone furoate ($C_{27}H_{30}Cl_2O_6$) in the portion of Mometasone Furoate taken:

$$\text{Result} = (R_U / R_S) \times (C_S / C_U) \times 100$$

R_U = peak response ratio of mometasone furoate to the internal standard from the *Sample solution*

R_S = peak response ratio of mometasone furoate to the internal standard from the *Standard solution*

C_S = concentration of USP Mometasone Furoate RS in the *Standard solution* (mg/mL)

C_U = concentration of Mometasone Furoate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 30 µg/g (Official 1-Jan-2018)

ORGANIC IMPURITIES

Standard stock solution: 10 mg/mL of USP

Mometasone Furoate RS in dichloromethane

Standard solution A (5%): 0.5 mg/mL of USP

Mometasone Furoate RS in dichloromethane from the *Standard stock solution*

Standard solution B (2%): 0.2 mg/mL of USP

Mometasone Furoate RS in dichloromethane, from the *Standard stock solution*

Standard solution C (1%): 0.1 mg/mL of USP

Mometasone Furoate RS in dichloromethane, from the *Standard stock solution*

Standard solution D (0.2%): 0.02 mg/mL of USP Mometasone Furoate RS in dichloromethane, from the *Standard stock solution*

Standard solution E (0.1%) 0.01 mg/mL of USP Mometasone Furoate RS in dichloromethane, from the *Standard stock solution*

Sample solution: 10 mg/mL of Mometasone Furoate in dichloromethane

Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 40 μ L

Developing solvent system: Chloroform and ethyl acetate (3:1)

Analysis

Samples: *Standard solutions* and *Sample solution*

Proceed as directed in the chapter. Examine the plate under short-wavelength UV light. Compare the intensities of any secondary spots from the *Sample solution* with those of the principal spots from the *Standard solutions*.

Acceptance criteria: No secondary spot from the *Sample solution* is larger or more intense than the principal spot from *Standard solution C*; and the sum of the intensities of the secondary spots from the *Sample solution* is NMT 2.0%.

SPECIFIC TESTS

• OPTICAL ROTATION, *Specific Rotation* (7815)

Sample solution: 5 mg/mL in dioxane

Acceptance criteria: +56° to +62°

• LOSS ON DRYING (731)

Analysis: Dry a sample at 105° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• USP REFERENCE STANDARDS (11)

USP Mometasone Furoate RS

Mometasone Furoate Cream

DEFINITION

Mometasone Furoate Cream is Mometasone Furoate in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of mometasone furoate ($C_{27}H_{30}Cl_2O_6$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.

• B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

Standard solution: 0.2 mg/mL of USP Mometasone Furoate RS in acetonitrile

Sample solution: 0.2 mg/mL of mometasone furoate from Cream in acetonitrile

Developing solvent system: Chloroform and ethyl acetate (3:1)

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• PROCEDURE

[NOTE—Protect from light.]

Diluent A: Tetrahydrofuran and glacial acetic acid (100:1)

Diluent B: Acetonitrile, water, and glacial acetic acid (50:50:1)

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
2	70	30
45	45	55
46	70	30
50	70	30

Internal standard solution: 1.4 mg/mL of diethyl phthalate in acetonitrile

Standard stock solution: 0.2 mg/mL of USP Mometasone Furoate RS in *Diluent A*

Standard solution: 0.05 mg/mL of mometasone furoate and 0.35 mg/mL of diethyl phthalate from equal quantities of the *Standard stock solution* and the *Internal standard solution*, in *Diluent B*

Sample solution: Transfer a portion of Cream, equivalent to 1.0 mg of mometasone furoate, to a 50-mL, screw-capped centrifuge tube. Add 5.0 mL of *Diluent A* and a few glass beads, and mix on a vortex mixer. Add 5.0 mL of *Internal standard solution*, and mix. Add 10.0 mL of *Diluent B*, mix on a vortex mixer for 1 min, and centrifuge for 10 min. Pass the aqueous phase through a polypropylene filter of 0.2- μ m pore size, discarding the first 1–2 mL of filtrate.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L60

Flow rate: 2 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for diethyl phthalate and mometasone furoate are 0.4 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 1.5 for the mometasone furoate peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mometasone furoate ($C_{27}H_{30}Cl_2O_6$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the mometasone furoate peak response to the diethyl phthalate peak response from the *Sample solution*

R_S = ratio of the mometasone furoate peak response to the diethyl phthalate peak response from the *Standard solution*

C_S = concentration of USP Mometasone Furoate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mometasone furoate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

IMPURITIES

• ORGANIC IMPURITIES

[NOTE—Protect from light.]

Diluent A, Solution A, Solution B, Mobile phase, and Standard stock solution: Prepare as directed in the Assay.

Diluent C: Acetonitrile, water, and glacial acetic acid (30:70:1)

System suitability solution: 0.1 µg/mL of USP

Mometasone Furoate RS from *Standard stock solution* in *Diluent C*

Blank solution: *Diluent C* and *Diluent A* (3:1)

Sample solution: Transfer a portion of Cream, equivalent to 2.0 mg of mometasone furoate, to a 50-mL, screw-capped centrifuge tube. Add 5.0 mL of *Diluent A* and a few glass beads, and mix on a vortex mixer. Add 15.0 mL of *Diluent C*, and mix. Centrifuge for 10 min. Pass the aqueous phase through a 0.2-µm polypropylene filter, discarding the first 1–2 mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L60

Column temperature: 25 ± 5°

Flow rate: 2 mL/min

Injection size: 50 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Relative standard deviation: NMT 10%

Analysis

Samples: *System suitability solution*, *Blank solution*, and *Sample solution*

[NOTE—Exclude any peak areas less than those from the *System suitability solution*. Also exclude any peaks with the same retention time as that observed in the *Blank solution*. Any peaks having a relative retention time of about 1.04 or 1.13 are controlled in the *Mometasone Furoate* monograph and, therefore, are not included in the total specified and unspecified impurities limit.]

Calculate the percentage of each impurity in the portion of Cream taken:

$$\text{Result} = (r_u/r_r) \times 100$$

r_u = peak response of each impurity from the *Sample solution*

r_r = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
9α-Chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.56	0.1
9α,21-Dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione	0.73	0.1
21-Chloro-17-hydroxy-16α-methylpregna-1,4-diene-3,11,20-trione 17-(2-furoate)	0.88	0.1

Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
21-Chloro-9β,11β-epoxy-17-hydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.94	1.0
Mometasone furoate	1.0	—
Unspecified individual impurity	—	0.2
Total specified and unspecified impurities	—	1.0

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Mometasone Furoate RS

Mometasone Furoate Ointment

DEFINITION

Mometasone Furoate Ointment is Mometasone Furoate in a suitable ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of mometasone furoate ($C_{27}H_{30}Cl_2O_6$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the Assay.

- **B. THIN-LAYER CHROMATOGRAPHY**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Standard solution: 0.6 mg/mL of USP Mometasone Furoate RS in methanol

Sample solution: Transfer the equivalent to 3 mg of mometasone furoate from Ointment to a 50-mL screw-capped centrifuge tube. Pipet 5.0 mL of methanol into the tube, and attach the cap. Heat in a steam bath until the Ointment completely melts, and shake vigorously until the Ointment resolidifies. Place in an ice-water bath for 10 min. Centrifuge, and filter a portion of the supernatant. Extract 1 mL of the filtrate with 1 mL of hexane, and use the lower phase.

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system A: Methanol

Developing solvent system B: Chloroform and ethyl acetate (3:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the spots to dry, and develop the chromatogram in *Developing solvent system A* until the solvent front has moved 2 cm from the origin. Remove the plate from the developing chamber, and air-dry. Develop the chromatogram in *Developing solvent system B* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the spots to air-dry. Examine the plate under short-wavelength UV light.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

PROCEDURE

[NOTE—Protect from light.]

Diluent A: Tetrahydrofuran and glacial acetic acid (100:1)

Diluent B: Acetonitrile, water, and glacial acetic acid (50:50:1)

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
2	70	30
45	45	55
46	70	30
50	70	30

Internal standard solution: 1.4 mg/mL of diethyl phthalate in acetonitrile

Standard stock solution: 0.2 mg/mL of USP Mometasone Furoate RS in *Diluent A*

Standard solution: 0.05 mg/mL of mometasone furoate and 0.35 mg/mL of diethyl phthalate from equal quantities of the *Standard stock solution* and the *Internal standard solution*, in *Diluent B*

Sample solution: Transfer a portion of Ointment, equivalent to 1.0 mg of mometasone furoate, to a 50-mL screw-capped centrifuge tube. Add 5.0 mL of *Diluent A* and a few glass beads, and mix on a vortex mixer. Add 5.0 mL of *Internal standard solution*, and mix. Add 10.0 mL of *Diluent B*, mix on a vortex mixer for 1 min, and centrifuge for 10 min. Pass the aqueous phase through a polypropylene filter of 0.2- μ m pore size, discarding the first 1–2 mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L60

Flow rate: 2 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for diethyl phthalate and mometasone furoate are 0.4 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 1.5 for the mometasone furoate peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mometasone furoate ($C_{27}H_{30}Cl_2O_6$) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the mometasone furoate peak response to the diethyl phthalate peak response from the *Sample solution*

R_S = ratio of the mometasone furoate peak response to the diethyl phthalate peak response from the *Standard solution*

C_S = concentration of USP Mometasone Furoate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mometasone furoate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

ORGANIC IMPURITIES

[NOTE—Protect from light.]

Diluent A, Solution A, Solution B, Mobile phase, and Standard stock solution: Prepare as directed in the Assay.

Diluent C: Acetonitrile, water, and glacial acetic acid (30:70:1)

System suitability solution: 0.1 μ g/mL of USP Mometasone Furoate RS from *Standard stock solution* in *Diluent C*

Sample solution: Transfer a portion of Ointment, equivalent to 2.0 mg of mometasone furoate, to a 50-mL screw-capped centrifuge tube. Add 5.0 mL of *Diluent A* and a few glass beads, and mix on a vortex mixer. Add 15.0 mL of *Diluent C*, and mix. Centrifuge for 10 min. Pass the aqueous phase through a polypropylene filter of 0.2- μ m pore size, discarding the first 1–2 mL of filtrate.

Blank solution: *Diluent C* and *Diluent A* (3:1)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L60

Column temperature: 25 \pm 5°

Flow rate: 2 mL/min

Injection size: 50 μ L

System suitability

Sample: *System suitability solution*

Suitability requirements

Relative standard deviation: NMT 10%

Analysis

Samples: *System suitability solution*, *Sample solution*, and *Blank solution*

[NOTE—Exclude any peak areas less than those from the chromatogram of the *System suitability solution*. Also exclude any peaks with the same retention time as that observed in the chromatogram of the *Blank solution*. Any peaks having a relative retention time of about 1.04 or 1.13 are controlled in the monograph for Mometasone Furoate, and therefore are not included in the total specified and unspecified impurities limit.]

Calculate the percentage of each impurity in the portion of Ointment taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
9 α -Chloro-11 β ,17,21-trihydroxy-16 α -methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.56	0.2
9 α ,21-Dichloro-11 β ,17-dihydroxy-16 α -methylpregna-1,4-diene-3,20-dione	0.73	0.2
21-Chloro-17-hydroxy-16 α -methylpregna-1,4-diene-3,11,20-trione 17-(2-furoate)	0.88	0.2

Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
21-Chloro-9 β ,11 β -epoxy-17-hydroxy-16 α -methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.94	1.0
Mometasone furoate	1.0	—
Unspecified individual impurity	—	0.2
Total specified and unspecified impurities	—	1.0

PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Mometasone Furoate RS

Mometasone Furoate Topical Solution**DEFINITION**

Mometasone Furoate Topical Solution is Mometasone Furoate in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of mometasone furoate ($C_{27}H_{30}Cl_2O_6$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the Assay.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**
Standard solution: 1 mg/mL of USP Mometasone Furoate RS in a mixture of chloroform and methanol (4:1)
Sample solution: Transfer the equivalent of 2 mg of mometasone furoate from Topical Solution to a 50-mL centrifuge tube. Add 10 mL of water. Extract the aqueous solution with 20 mL of chloroform. Remove the chloroform layer, dry over anhydrous sodium sulfate, and filter through a cotton pledget. Repeat the chloroform extraction, and combine the dried extracts. Evaporate the chloroform solution to dryness on a steam bath under a stream of nitrogen. Allow the sample specimen to cool to room temperature. Dissolve the residue in a mixture of chloroform and methanol (4:1) to obtain 1 mg/mL of *Sample solution*.
Application volume: 20 μ L
Developing solvent system: Chloroform and ethyl acetate (3:1)
Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to the that of the *Standard solution*.

ASSAY• **PROCEDURE**

[NOTE—Protect from light.]

Diluent: Acetonitrile, water, and glacial acetic acid (50:50:1)

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
2	70	30
45	45	55
46	70	30
50	70	30

Standard solution: 0.1 mg/mL of USP Mometasone Furoate RS in *Solution B*

Sample solution: Transfer a portion of Topical Solution, equivalent to about 2.5 mg of mometasone furoate, to a 25-mL flask. Dilute with *Diluent* to volume, and mix. Pass a portion of the solution through a polypropylene filter of 0.2- μ m pore size, discarding the first 1–2 mL of filtrate.

Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L60

Flow rate: 2 mL/min

Injection size: 50 μ L

System suitability

Sample: *Standard solution*

Suitability requirements:

Tailing factor: NMT 1.5 for the mometasone furoate peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mometasone furoate ($C_{27}H_{30}Cl_2O_6$) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mometasone Furoate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mometasone furoate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES• **ORGANIC IMPURITIES**

[NOTE—Protect from light.]

Diluent, Solution A, Solution B, Mobile phase, Standard solution, and Sample solution: Prepare as directed in the Assay.

System suitability solution: 0.1 μ g/mL of USP Mometasone Furoate RS from *Standard solution* in *Diluent*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-μm packing L60

Column temperature: 25 ± 5°

Flow rate: 2 mL/min

Injection size: 50 μL

System suitabilitySample: *System suitability solution***Suitability requirements**

Relative standard deviation: NMT 10%

AnalysisSamples: *Diluent*, *System suitability solution*, and *Sample solution*

[NOTE—Exclude any peak areas less than that of the *System suitability solution*. Also, exclude any peaks with the same retention times as those observed in the *Diluent*. Any peaks having a relative retention time of 1.04 or 1.13 are controlled in the *Mometasone Furoate* monograph, and therefore are not included in the total specified and unspecified impurities limit.]

Calculate the percentage of each impurity in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
9α-Chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.56	0.3
9α,21-Dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione	0.73	0.1
21-Chloro-17-hydroxy-16α-methylpregna-1,4-diene-3,11,20-trione 17-(2-furoate)	0.88	0.1
21-Chloro-9β,11β-epoxy-17-hydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)	0.94	1.0
Mometasone furoate	1.0	—
Unspecified individual impurity	—	0.5
Total specified and unspecified impurities	—	2.0

SPECIFIC TESTS

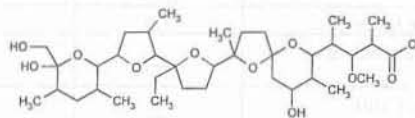
- MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.
- PH** (791): 4.0–5.0

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Mometasone Furoate RS

MonensinC₃₆H₆₂O₁₁(monensin A) 670.87C₃₅H₆₀O₁₁(monensin B) 656.84C₃₇H₆₄O₁₁(monensin C) 684.90

Monensin.

Stereoisomer of 2-[2-ethyloctahydro-3'-methyl-5'-[tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl][2,2'-bifuran-5-yl]-9-hydroxy-β-methoxy-α,γ,2,8-tetramethyl-1,6-dioxaspiro[4.5]decan-7-butanoic acid [17090-79-8].

» Monensin is a mixture of antibiotic substances produced by the growth of *Streptomyces cinnamonensis*. It has a potency of not less than 110 μg of monensin per mg.

Packaging and storage—Preserve in well-closed containers. Avoid moisture and excessive heat.

Labeling—Label it to indicate that it is for veterinary use only. Label it also to state that it is for manufacturing, processing, or repackaging.

USP Reference standards (11)—

USP Monensin Sodium RS

USP Narasin RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for monensin A and a minor peak for monensin B, the retention times of which correspond to those exhibited in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

Loss on drying (731)—Dry it in vacuum at 60° for 2 hours: it loses not more than 10% of its weight.

Content of monensin A and B activity—Using the results of the calculations in the *Assay*, calculate the percentage of monensin A activity in the Monensin under test by the formula:

$$100A / P$$

in which *A* is the potency, in μg per mg, of monensin A in the Monensin under test, as determined in the *Assay*, and *P* is the potency, in μg of monensin, in each mg of the Monensin under test, as determined in the *Assay*: not less than 90% is found. Calculate the percentage of monensin A activity plus monensin B activity in the Monensin under test by the formula:

$$100(A + B) / P$$

in which *B* is the potency, in μg per mg, of monensin B in the Monensin under test, as determined in the *Assay*, and the other terms are as defined above: not less than 95% is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of methanol, water, and glacial acetic acid (94:6:0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Neutralized methanol—Add 1 g of sodium bicarbonate to 4 liters of methanol, mix, and filter.

Diluent—Prepare a mixture of methanol and water (9:1).

Derivatizing reagent—Dissolve 3 g of vanillin in a mixture of 95 mL of methanol and 2 mL of sulfuric acid. [Caution—To avoid splattering, add the sulfuric acid carefully and slowly with a pipet; do not pour. Allow the mixture of methanol and sulfuric acid to cool before adding vanillin.]

Standard preparation—Dissolve an accurately weighed quantity of USP Monensin Sodium RS quantitatively in methanol to obtain a solution containing the equivalent of 1000 µg of monensin per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Diluent* to obtain a solution containing 20.0 µg of monensin per mL.

Assay preparation—Transfer about 500 mg of Monensin, accurately weighed, to a 250-mL flask, add 200.0 mL of *Diluent*, and shake by mechanical means for 1 hour. Allow the solids to settle, and dilute an accurately measured volume of the supernatant quantitatively with *Diluent* to obtain a solution containing about 20 µg of monensin per mL.

Resolution solution—Prepare a solution in *Neutralized methanol* containing about 1 mg of USP Monensin Sodium RS and 3 mg of USP Narasin RS per mL. Transfer 2 mL of this solution to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 4.6-mm × 25-cm column that contains packing L1 and the outlet of which is attached to a tee, the opposing arm of which is attached to a tube from which is pumped the *Derivatizing reagent*, and the outlet of which is connected to a 2-mL postcolumn reaction coil maintained at 98°. The outlet of the reaction coil is connected to a detector set at 520 nm. The *Mobile phase* and the *Derivatizing reagent* flow at the rate of about 0.7 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.9 for monensin B, 1.0 for monensin A, 1.3 for narasin A, and 1.5 for narasin I, the resolution, *R*, between the monensin B peak and the monensin A peak is not less than 1.25, and between the monensin A peak and the narasin A peak is not less than 3.5. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the tailing factor is not more than 1.4, and the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—After use, flush the system with methanol.]

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 200 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks, including a peak for monensin C/D, if present, at a retention time of about 1.1 relative to that of the main monensin A peak in the chromatogram obtained from the *Assay preparation*. Calculate the quantity, in µg, of monensin A in each mg of the Monensin taken by the formula:

$$(CFD / 100,000W)(r_u / r_s)$$

in which *C* is the concentration, in µg per mL, of monensin activity in the *Standard preparation*, based on the quantity of USP Monensin Sodium RS taken, its designated potency, in µg per mg, and the extent of dilution, *F* is the designated percentage of monensin A in USP Monensin Sodium RS, *D* is the dilution factor used in preparing the *Assay preparation*, *W* is the quantity, in g, of Monensin taken to prepare the *Assay preparation*, and *r_u* and *r_s* are the monensin A peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in µg, of monensin B in each mg of the Monensin taken by the same formula, except that *r_u* is the monensin B peak response obtained from the *Assay preparation* and *r_s* is the

monensin A peak response obtained from the *Standard preparation*. Calculate the quantity, in µg, of monensin C/D in each mg of the Monensin taken by the same formula, except that *r_u* is the monensin C/D peak response obtained from the *Assay preparation*. Calculate the potency, in µg of monensin, in each mg of the Monensin taken by the formula:

$$A + 0.28B + 1.5C / D$$

in which *A* is the quantity, in µg, of monensin A in each mg of the Monensin taken, as calculated above, and *B* is the quantity, in µg, of monensin B in each mg of the Monensin taken, and *C/D* is the quantity, in µg, of monensin C/D in each mg of Monensin taken, as calculated above.

Monensin Granulated

» Monensin Granulated contains Monensin mixed with suitable diluents, carriers, and inactive ingredients prepared in a granulated form that is free-flowing and free from aggregates. It may contain added Monensin Sodium. It contains not less than 140 mg of monensin per g.

Packaging and storage—Preserve in well-closed containers. Avoid moisture and excessive heat.

Labeling—Label it to indicate that it is for veterinary use only. Label it also to state that it is for manufacturing, processing, or repackaging.

USP Reference standards (11)—

USP Monensin Sodium RS

USP Narasin RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for monensin A and a minor peak for monensin B, the retention times of which correspond to those exhibited in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

Loss on drying (731)—Dry it in vacuum at 60° for 2 hours: it loses not more than 10% of its weight.

Content of monensin A and B activity—Using the results of the calculations in the *Assay*, calculate the percentage of monensin A activity in the Monensin Granulated under test by the formula:

$$100A / P$$

in which *A* is the potency, in µg per mg, of monensin A in the Monensin Granulated under test, as determined in the *Assay*, and *P* is the potency, in µg of monensin, in each mg of the Monensin Granulated under test, as determined in the *Assay*: not less than 90% is found. Calculate the percentage of monensin A activity plus monensin B activity in the Monensin Granulated under test by the formula:

$$100(A + B) / P$$

in which *B* is the potency, in µg per mg, of monensin B in the Monensin Granulated under test, as determined in the *Assay*, and the other terms are as defined above: not less than 95% is found.

Assay—

Mobile phase, *Neutralized methanol*, *Diluent*, *Derivatizing reagent*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay* under Monensin.

Assay preparation—Transfer about 5 g of Monensin Granulated, accurately weighed, to a 250-mL flask, add 200.0 mL

of *Diluent*, and shake by mechanical means for 1 hour. Allow the solids to settle, and dilute an accurately measured volume of the supernatant quantitatively with *Diluent* to obtain a solution containing about 20 µg of monensin per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Monensin*. Calculate the quantity, in mg, of monensin A in each g of the Monensin Granulated taken by the formula:

$$(CFD / 100,000W)(r_u / r_s)$$

in which C is the concentration, in µg per mL, of monensin activity in the *Standard preparation*, based on the quantity of USP Monensin Sodium RS taken, its designated potency, in µg per mg, and the extent of dilution, F is the designated percentage of monensin A in USP Monensin Sodium RS, D is the dilution factor used in preparing the *Assay preparation*, W is the quantity, in g, of Monensin Granulated taken to prepare the *Assay preparation*, and r_u and r_s are the monensin A peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of monensin B in each g of the Monensin Granulated taken by the same formula, except that r_u is the monensin B peak response obtained from the *Assay preparation* and r_s is the monensin A peak response obtained from the *Standard preparation*. Calculate the quantity, in mg, of monensin C/D in each g of the Monensin Granulated taken by the same formula, except that r_u is the monensin C/D peak response obtained from the *Assay preparation*. Calculate the potency, in µg of monensin, in each mg of the Monensin Granulated taken by the formula:

$$A + 0.28B + 1.5C / D$$

in which A is the quantity, in mg, of monensin A in each g of the Monensin Granulated taken, as calculated above, and B is the quantity, in mg, of monensin B in each g of the Monensin Granulated taken, and C/D is the quantity, in mg, of monensin C/D in each g of Monensin Granulated taken, as calculated above.

Monensin Premix

» Monensin Premix contains Monensin Granulated mixed with suitable diluents and inactive ingredients. It contains the equivalent of not less than 85.0 percent and not more than 115.0 percent of the labeled amount of monensin.

Packaging and storage—Preserve in well-closed containers. Avoid moisture and excessive heat.

Labeling—Label it to indicate that it is for veterinary use only. The label bears the statement "Do not feed undiluted."

USP Reference standards (11)—

USP Monensin Sodium RS

USP Narasin RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for monensin A and a minor peak for monensin B, the retention times of which correspond to those exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Loss on drying (731)—Dry it in vacuum at 60° for 2 hours: it loses not more than 10% of its weight.

Assay—

Mobile phase, Neutralized methanol, Diluent, Derivatizing reagent, Standard preparation, Resolution solution, and Chro-

matographic system—Proceed as directed in the *Assay* under *Monensin*.

Assay preparation—Transfer about 5 g of Premix, accurately weighed, to a 250-mL flask, add 200.0 mL of *Diluent*, and shake by mechanical means for 1 hour. Allow the solids to settle, and dilute an accurately measured volume of the clear supernatant quantitatively with *Diluent* to obtain a solution containing about 20 µg of monensin per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Monensin*. Calculate the quantity, in mg, of monensin A in each g of the Premix taken by the formula:

$$(CFD / 100,000W)(r_u / r_s)$$

in which C is the concentration, in µg per mL, of monensin activity in the *Standard preparation*, based on the quantity of USP Monensin Sodium RS taken, its designated potency, in µg per mg, and the extent of dilution, F is the designated percentage of monensin A in USP Monensin Sodium RS, D is the dilution factor used in preparing the *Assay preparation*, W is the quantity, in g, of Premix taken to prepare the *Assay preparation*, and r_u and r_s are the monensin A peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of monensin B in each g of the Premix taken by the same formula, except that r_u is the monensin B peak response obtained from the *Assay preparation* and r_s is the monensin A peak response obtained from the *Standard preparation*. Calculate the quantity, in mg, of monensin C/D in each g of the Premix taken by the same formula, except that r_u is the monensin C/D peak response obtained from the *Assay preparation*. Calculate the potency, in mg of monensin, in each g of the Premix taken by the formula:

$$A + 0.28B + 1.5C / D$$

in which A is the quantity, in mg, of monensin A in each g of the Premix taken, as calculated above, and B is the quantity, in mg, of monensin B in each g of the Premix taken, and C/D is the quantity, in mg, of monensin C/D in each g of Premix taken, as calculated above.

Monensin Sodium

C₃₆H₆₁NaO₁₁ (monensin A sodium) 692.85

C₃₅H₅₉NaO₁₁ (monensin B sodium) 678.83

C₃₇H₆₃NaO₁₁ (monensin C sodium) 706.88

Monensin, sodium salt.

Stereoisomer of 2-[2-ethyloctahydro-3'-methyl-5'-

[tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl][2,2'-bifuran-5-yl]-9-hydroxy-β-methoxy-α, γ,2,8-tetramethyl-1,6-dioxaspiro[4.5]decan-7-butanoic acid sodium salt [22373-78-0].

» Monensin Sodium has a potency of not less than 800 µg per mg.

Packaging and storage—Preserve in well-closed containers. Avoid moisture and excessive heat.

Labeling—Label it to indicate that it is for veterinary use only. Label it also to state that it is for manufacturing, processing, or repackaging.

USP Reference standards (11)—

USP Monensin Sodium RS

USP Narasin RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for monensin A and a minor peak for monensin B, the retention times of which correspond to those exhibited in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Loss on drying (731)—Dry it in vacuum at 60° for 3 hours; it loses not more than 4% of its weight.

Content of monensin A and B activity—Using the results of the calculations in the Assay, calculate the percentage of monensin A activity in the Monensin Sodium under test by the formula:

$$100A / P$$

in which *A* is the potency, in mg per g, of monensin A in the Monensin Sodium under test, as determined in the Assay, and *P* is the potency, in mg of monensin, in each g of the Monensin Sodium under test, as determined in the Assay; not less than 90% is found. Calculate the percentage of monensin A activity plus monensin B activity in the Monensin Sodium under test by the formula:

$$100(A + B) / P$$

in which *B* is the potency, in mg per g, of monensin B in the Monensin Sodium under test, as determined in the Assay, and the other terms are as defined above; not less than 95% is found.

Assay—

Mobile phase, Neutralized methanol, Diluent, Derivatizing reagent, Standard preparation, Resolution solution, and Chromatographic system—Proceed as directed in the Assay under Monensin.

Assay preparation—Transfer about 100 mg of Monensin Sodium, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume. If necessary, to achieve complete dissolution, sonicate for about 1 minute, and mix. Dilute an accurately measured volume of this solution quantitatively with *Diluent* to obtain a solution containing about 20 µg of monensin per mL.

Procedure—Proceed as directed for *Procedure* in the Assay under Monensin. Calculate the quantity, in mg, of monensin A in each g of the Monensin Sodium taken by the formula:

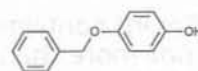
$$(CFD / 100,000W)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of monensin activity in the *Standard preparation*, based on the quantity of USP Monensin Sodium RS taken, its designated potency, in µg per mg, and the extent of dilution; *F* is the designated percentage of monensin A in USP Monensin Sodium RS; *D* is the dilution factor used in preparing the *Assay preparation*; *W* is the quantity, in g, of Monensin Sodium taken to prepare the *Assay preparation*; and *r_U* and *r_S* are the monensin A peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of monensin B in each g of the Monensin Sodium taken by the same formula, except that *r_U* is the monensin B peak response obtained from the *Assay preparation*, and *r_S* is the monensin A peak response obtained from the *Standard preparation*. Calculate the quantity, in mg, of monensin C/D in each g of the Monensin Sodium taken by the same formula, except that *r_U* is the monensin C/D peak response obtained from the *Assay preparation*. Calculate the potency, in mg of monensin, in each g of the Monensin Sodium taken by the formula:

$$A + 0.28B + 1.5C / D$$

in which *A* is the quantity, in mg, of monensin A in each g of the Monensin Sodium taken, as calculated above; *B* is the quantity, in mg, of monensin B in each g of the Monensin Sodium taken; and *C/D* is the quantity, in mg, of monensin C/D in each g of Monensin Sodium taken, as calculated above.

Monobenzene



$C_{13}H_{12}O_2$ 200.23

Phenol, 4-(phenylmethoxy)-.

p-(Benzyloxy)phenol [103-16-2].

» Monobenzene, dried at 105° for 3 hours, contains not less than 98.0 percent and not more than 102.0 percent of $C_{13}H_{12}O_2$.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to temperatures above 30°.

USP Reference standards (11)—

USP Monobenzene RS

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 10 µg per mL.

Medium: methanol.

Absorptivities at 292 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: Transfer about 500 mg of Monobenzene, previously dried, to a 150-mL flask fitted with a reflux condenser, employing a suitable glass joint. Add 5 mL of pyridine and 3 mL of acetic anhydride, reflux for 10 minutes, and cool. Add 100 mL of water and 6 mL of acetone to the flask, and insert a stopper. Cool the contents of the flask in a refrigerator for 1 hour, collect the precipitate in a sintered-glass crucible, and wash the precipitate with water until no odor of pyridine remains. Dry the precipitate for 16 hours in a vacuum desiccator over phosphorus pentoxide. The monobenzene acetate so obtained melts between 110° and 113° when determined as directed for *Class I* (see *Melting Range or Temperature* (741)).

Melting range, Class I (741): between 117° and 120°.

Loss on drying (731)—Dry it at 105° for 3 hours; it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.5%.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Monobenzene RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 40 µg per mL.

Assay preparation—Transfer about 100 mg of Monobenzene, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Pipet 4 mL of this solution into a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—With a suitable spectrophotometer, using methanol as a blank, concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the wavelength of maximum absorbance at about 292 nm. Calculate the quantity, in mg, of $C_{13}H_{12}O_2$ in the portion of Monobenzene taken by the formula:

$$2500C(A_U / A_S)$$

in which *C* is the concentration, in mg per mL, of USP Monobenzene RS in the *Standard preparation*; and *A_U* and *A_S* are the absorbances obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Monobenzene Cream

» Monobenzene Cream contains not less than 94.0 percent and not more than 106.0 percent of the labeled amount of monobenzene ($C_{13}H_{12}O_2$).

Packaging and storage—Preserve in tight containers, and avoid exposure to temperatures higher than 30°.

USP Reference standards (11)—
USP Monobenzene RS

Identification—Transfer a quantity of Cream, equivalent to about 500 mg of monobenzene, to a centrifuge bottle, add 100 mL of water, and shake until the cream is completely dispersed. Centrifuge the suspension, decant the supernatant, wash the residue with water, again centrifuge, and decant the water. Transfer the residue to a separator with the aid of water, and adjust the volume to about 100 mL. Extract with four 25-mL portions of chloroform, filtering the extracts through a pledget of cotton into a 150-mL flask. Evaporate the chloroform in a current of warm air, and add 5 mL of pyridine and 3 mL of acetic anhydride to the dry residue. Connect the flask to a reflux condenser, reflux for 10 minutes, cool, and proceed as directed in *Identification* test C under *Monobenzene*, beginning with "Add 100 mL of water." It meets the requirements of *Identification* test C under *Monobenzene*.

Assay—

Standard preparation—Prepare as directed in the *Assay* under *Monobenzene*.

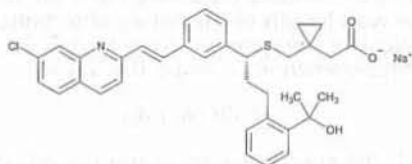
Assay preparation—Transfer an accurately weighed portion of Cream, equivalent to about 200 mg of monobenzene, to a suitable container, add 100 mL of methanol, and shake for about 30 minutes. Transfer the mixture to a 200-mL volumetric flask. Rinse the container with two 25-mL portions of methanol, and add the rinsings to the 200-mL volumetric flask. Dilute with methanol to volume, mix, and filter, discarding the first 20 mL of the filtrate. Pipet 4 mL of this solution into a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—Proceed as directed in the *Assay* under *Monobenzene*, but use the *Assay preparation* under *Monobenzene Cream*. Calculate the quantity, in mg, of monobenzene ($C_{13}H_{12}O_2$) in the portion of Cream taken by the formula:

$$5000C(A_U / A_S)$$

in which *C* is the concentration, in mg per mL, of USP Monobenzene RS in the *Standard preparation* and A_U and A_S are the absorbances obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Montelukast Sodium



$C_{35}H_{35}ClINNaO_3S$ 608.17
Cyclopropaneacetic acid, 1-[[[1-[3-[2-(7-chloro-2-quinolyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]-, sodium salt, [R-, (E)-];

Sodium 1-[[[1-[(R)-m-[(E)-2-(7-chloro-2-quinolyl)vinyl]-α-[o-(1-hydroxy-1-methylethyl)phenethyl]benzyl]thio]-methyl]cyclopropaneacetate [151767-02-1].

$C_{35}H_{35}ClINNaO_3S$ 586.18
Montelukast [158966-92-8].

DEFINITION

Montelukast Sodium contains NLT 98.0% and NMT 102.0% of $C_{35}H_{35}ClINNaO_3S$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

A. INFRARED ABSORPTION (197)

[NOTE—Methods described under *Infrared Absorption* (197K), (197M), or (197A) may be used.]

B. IDENTIFICATION TESTS—GENERAL, Sodium (191)

Sample: 100 mg

Analysis: Ignite the *Sample* in a crucible until an almost white residue is obtained. Take up the residue in 2 mL of water, and filter.

Acceptance criteria: The filtrate meets the requirements of the pyroantimonate precipitate test.

C. Meets the requirements of the test for Enantiomeric Purity.

ASSAY

[NOTE—Avoid exposure of the samples to light. Use low-actinic glassware.]

PROCEDURE

Solution A: Add 1.5 mL of trifluoroacetic acid to 1 L of water.

Solution B: Add 1.5 mL of trifluoroacetic acid to 1 L of acetonitrile.

Mobile phase: See *Table 1*. Return to original conditions and re-equilibrate the column.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
3.0	60	40
16.0	49	51

Diluent: Methanol and water (9:1)

Standard solution: 0.13 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*

Sample solution: 0.1 mg/mL of Montelukast Sodium in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 238 nm

Column: 4.6-mm × 5-cm; 1.8-μm packing L11

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection size: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of montelukast sodium ($C_{35}H_{35}ClINNaO_3S$) in the portion of Montelukast Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

M_{r1} = molecular weight of montelukast sodium, 608.17

M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

Delete the following:

• HEAVY METALS

Diluent: Acetone and water (4:1)

Sample solution: Dissolve 0.50 g of Montelukast Sodium in 20 mL of Diluent.

Reference solution: Dilute 0.5 mL of the *Standard Lead Solution*, prepared as directed under *Heavy Metals* (231), with Diluent to 20 mL.

Blank solution: 20 mL of the Diluent

Analysis: To each solution, add 2 mL of pH 3.5 Acetate Buffer, prepared as directed under *Heavy Metals* (231). Mix, and add to 1.2 mL of thioacetamide–glycerin base TS. Mix immediately, and allow to stand for 2 min. Pass the solutions through a membrane filter of 0.45- μ m pore size. Compare the spots on the filters obtained from the different solutions: the brownish-black color of the spot resulting from the *Sample solution* is not more intense than that of the spot resulting from the *Reference solution*. The test is invalid if the *Reference solution* does not show a brownish-black color compared to the *Blank solution*.

Acceptance criteria: NMT 10 ppm • (Official 1-Jan-2018)

• ORGANIC IMPURITIES

[NOTE—Avoid exposure of the samples to light. Use low-actinic glassware.]

Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the Assay.

Impurity solution: 1 mg/mL of USP Montelukast for Peak Identification RS in Diluent

System suitability solution: Transfer 1 mL of the *Impurity solution* to a colorless glass vial, and expose to ambient light for approximately 20 min to generate the *cis*-isomer of montelukast.

Sample solution: 1 mg/mL of Montelukast Sodium in Diluent

Sensitivity solution: 0.5 μ g/mL of Montelukast Sodium in Diluent from the *Sample solution*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.5 between the *cis*-isomer and montelukast; NLT 1.5 between montelukast and the methylketone impurity, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Montelukast Sodium taken:

$$\text{Result} = (r_u/r_T) \times 100$$

r_u = peak response of each impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See Table 2.

Reporting level for impurities: 0.05%

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sulfoxide impurity ^a	0.4	0.2
<i>Cis</i> -isomer ^b	0.8	0.15
Michael Adducts 1 ^c and 2 ^d	0.9	0.15*
Montelukast	1.0	—
Methylketone impurity ^e	1.2	0.15
Methylstyrene impurity ^f	1.9	0.3
Any other individual impurity	—	0.10
Total impurities	—	0.6

* These two impurities are not resolved by the method and need to be integrated together to determine conformance.

^a 1-[[[1-[[3-[(E)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^b 1-[[[1-[(1R)-1-[3-[(2Z)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^c 1-[[[1-[(1R)-1-[3-[(1R)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfonyl]-2-(7-chloroquinolin-2-yl)ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^d 1-[[[1-[(1R)-1-[3-[(1S)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfonyl]-2-(7-chloroquinolin-2-yl)ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^e 1-[[[1-[(1R)-3-(2-Acetylphenyl)-1-[3-[(E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^f 1-[[[1-[(1R)-1-[3-[(E)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

• ENANTIOMERIC PURITY

[NOTE—Avoid exposure of the samples to light. Use low-actinic glassware.]

Solution A: 2.3 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 5.7.

Solution B: Methanol and acetonitrile (60:40)

Mobile phase: See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	70	30
30	60	40
35	60	40

Diluent: Acetonitrile and water (1:1)

System suitability solution: 0.1 mg/mL of USP Montelukast Racemate RS in Diluent

Sample solution: 1 mg/mL of Montelukast Sodium in Diluent

Sensitivity solution: 1 μ g/mL of Montelukast Sodium in Diluent from the *Sample solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm \times 15-cm; 5- μ m packing L41

Column temperature: 30°

Flow rate: 0.9 mL/min

Injection size: 10 μ L

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—The relative retention times are 1.0 for montelukast, which is the *R*-enantiomer, and 0.7 for the *S*-enantiomer.]

Suitability requirements

Resolution: NLT 2.9 between the *S*-enantiomer and montelukast, *System suitability solution*

Signal-to-noise ratio: NLT 10 for the montelukast peak, *Sensitivity solution*

Analysis**Sample:** *Sample solution*Calculate the percentage of *S*-enantiomer in the portion of Montelukast Sodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 r_U = peak response of the *S*-enantiomer from the *Sample solution* r_T = sum of the peak responses of the *S*-enantiomer and montelukast from the *Sample solution*Acceptance criteria: NMT 0.2% of the *S*-enantiomer**SPECIFIC TESTS**

- **WATER DETERMINATION, Method 1a (921):** NMT 4.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at room temperature.
- **USP REFERENCE STANDARDS (11)**
 - USP Montelukast Sodium RS
 - USP Montelukast Dicyclohexylamine RS
 $C_{35}H_{36}ClNO_3S \cdot C_{12}H_{23}N$ 767.50
 - USP Montelukast Racemate RS
 - USP Montelukast for Peak Identification RS
(montelukast containing sulfoxide impurity, michael adducts 1 and 2, methylketone impurity, and methylstyrene impurity)

Montelukast Sodium Oral Granules**DEFINITION**Montelukast Sodium Oral Granules contain Montelukast Sodium equivalent to NLT 90.0% and NMT 108.0% of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$).

[NOTE—Avoid exposure of samples containing montelukast to light.]

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION (197U)**
 - Diluent:** Methanol and water (3:1)
 - Standard solution:** 3.3 µg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*
 - Sample stock solution:** Nominally 0.02 mg/mL of montelukast prepared as follows. Transfer the contents of one packet to a suitable volumetric flask, add 66% of the flask volume of *Diluent*, shake well, and sonicate for 15 min with occasional shaking. Cool to room temperature, dilute with *Diluent* to volume, and mix well.
 - Sample solution:** Nominally 2 µg/mL of montelukast in *Diluent* from the *Sample stock solution*. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size or centrifuge to obtain a clear solution.
 - Wavelength range:** 210–400 nm
 - Acceptance criteria:** The *Sample solution* exhibits maxima only at the same wavelengths as the *Standard solution*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY**PROCEDURE**

Diluent: Methanol and water (3:1)
Solution A: 0.2% (v/v) Trifluoroacetic acid in water
Solution B: Methanol and acetonitrile (3:2)
Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	48	52
5	45	55
12	45	55
22	25	75
23	25	75
25	48	52
30	48	52

Standard solution: 0.33 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent***System suitability solution:** Transfer 10 mL of the *Standard solution* to a clear 10-mL volumetric flask, add 4 µL of hydrogen peroxide, and mix well. Expose the flask for at least 4 h to ambient light or 10 min to a 4 klx cool white light. [NOTE—Montelukast is partially converted to the *cis*-isomer under these conditions.]**Sensitivity solution:** 0.33 µg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent* from the *Standard solution***Sample solution:** Nominally 0.24 mg/mL of montelukast prepared as follows. Transfer the equivalent of 60 mg of montelukast from the contents of the packets (NLT 15) to a 500-mL volumetric flask, and add 250 mL of *Diluent*. Shake well and sonicate for 30 min, with occasional shaking. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size or centrifuge to obtain a clear solution.**Chromatographic system**(See Chromatography (621), *System Suitability*.)**Mode:** LC**Detector:** UV 255 nm**Columns****Guard:** 3.0-mm × 4-mm; packing L11**Analytical:** 4.6-mm × 10-cm; 3-µm packing L11**Column temperature:** 50°**Flow rate:** 1.5 mL/min**Injection volume:** 20 µL**Run time:** 2 times the retention time of montelukast**System suitability****Samples:** *Standard solution*, *System suitability solution*, and *Sensitivity solution*[NOTE—The relative retention times for the *cis*-isomer and montelukast are about 0.92 and 1.0, respectively.]**Suitability requirements****Resolution:** NLT 1.5 between the *cis*-isomer and montelukast, *System suitability solution***Relative standard deviation:** NMT 2.0% for five injections, *Standard solution***Signal-to-noise ratio:** NLT 10, *Sensitivity solution***Analysis****Samples:** *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) in the portion of Oral Granules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL) C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL) M_{r1} = molecular weight of montelukast, 586.18 M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: 90.0%–108.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Medium: 0.5% (w/v) Sodium dodecyl sulfate in water; 900 mL. Do not deaerate.

Apparatus 1: 100 mesh; 50 rpm

Time: 15 min

Solution A: 0.2% (v/v) Trifluoroacetic acid in water

Solution B: 0.2% (v/v) Trifluoroacetic acid in acetonitrile

Mobile phase: *Solution A* and *Solution B* (1:1)

Standard stock solution: 0.33 mg/mL of USP Montelukast Dicyclohexylamine RS in methanol (equivalent to 0.25 mg/mL of montelukast)

Standard solution: (L/900) mg/mL of montelukast in *Medium* from the *Standard stock solution*, where L is the label claim in mg/packet of montelukast

Sample solution: Place the entire contents of one packet in the basket. At the appropriate time point, pass a portion of the solution under test through a suitable filter to obtain a clear solution. Discard the first 10 mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 389 nm

Column: 3.0-mm × 10-cm; 5-μm packing L11

Column temperature: 50°

Flow rate: 0.9 mL/min

Injection volume: 25 μL

Run time: 1.5 times the retention time of montelukast

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast (C₃₅H₃₆ClNO₃S) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of montelukast from the *Sample solution*

r_S = peak response of montelukast from the *Standard solution*

C_S = concentration of montelukast in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/packet)

Tolerances: NLT 85% (Q) of the labeled amount of montelukast (C₃₅H₃₆ClNO₃S) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.5% (w/v) Sodium dodecyl sulfate in water; 900 mL

Apparatus 1: 100 mesh; 50 rpm

Time: 15 min

Solution A: 0.07 g/L of monobasic sodium phosphate

Solution B: Acetonitrile

Mobile phase: *Solution A* and *Solution B* (45:55). Add 1.33 mL/L of triethylamine and adjust with phosphoric acid to a pH of 6.7.

Standard stock solution: 0.1 mg/mL of montelukast from montelukast sodium hydrate prepared as follows. Transfer a suitable amount of montelukast sodium hydrate to an appropriate volumetric flask. Dissolve in 4% of the flask volume of methanol and dilute with *Medium* to volume. Determine the water content of montelukast sodium hydrate at the time of use.

Standard solution: 0.004 mg/mL of montelukast in *Medium* from the *Standard stock solution*

Sample solution: Place the entire contents of one packet in the basket. At the appropriate time point, centrifuge a portion of the solution under test.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 5-cm; 1.8-μm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 100 μL

Run time: 1.5 times the retention time of montelukast

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast (C₃₅H₃₆ClNO₃S) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of montelukast in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/packet)

Tolerances: NLT 80% (Q) of the labeled amount of montelukast (C₃₅H₃₆ClNO₃S) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Solution A, Solution B, Mobile phase, and System suitability: Proceed as directed in *Dissolution Test 1*.

Standard solution: 26.4 μg/mL of USP Montelukast Dicyclohexylamine RS in methanol

Sample solution: Nominally 0.02 mg/mL of montelukast prepared as follows. Transfer the contents of one packet to a suitable volumetric flask, add 66% of the flask volume of methanol, shake well, and sonicate for 15 min with occasional shaking. Cool to room temperature, dilute with methanol to volume, and mix well. Pass a portion of the resulting solution through a suitable filter of 0.45-μm pore size or centrifuge to obtain a clear solution.

Chromatographic system: Proceed as directed in *Dissolution Test 1*, except use an *Injection volume* of 5 μL.

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of montelukast (C₃₅H₃₆ClNO₃S) in the packet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of montelukast, 586.18

M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Diluent, Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, Sensitivity solution, Sample solution, Chromatographic system,

and **System suitability:** Proceed as directed in the Assay.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Oral Granules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*

r_S = peak response of montelukast from the *Standard solution*

C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of montelukast, 586.18

M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

F = relative response factor (see Table 2)

Acceptance criteria: See Table 2. Disregard any peak with an area less than that of the *Sensitivity solution*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Sulfoxide impurity ^{a,b}	0.45	1.0	0.8
Montelukast ketone impurity ^c	0.71	1.7	0.2
<i>cis</i> -Isomer ^d	0.92	1.0	0.2
Montelukast	1.0	—	—
Methylketone impurity ^{e,f}	1.04	—	—
Michael adduct 1 ^{g,e}	1.16	—	—
Michael adduct 2 ^{h,e}	1.18	—	—
Methylstyrene impurity ^{i,e}	1.55	—	—

^a These two impurities are not resolved by the method and need to be integrated together to determine conformance.

^b 1-[[[1-(3-[(E)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl)-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^c (E)-1-[3-[2-(7-Chloroquinolin-2-yl)vinyl]phenyl]-3-[2-(2-hydroxypropan-2-yl)phenyl]propan-1-one.

^d 1-[[[1-(R)-1-[3-[(Z)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^e This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.

^f 1-[[[1-(R)-3-(2-Acetylphenyl)-1-[3-[(E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^g 1-[[[1-(R)-1-[3-[(1R)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfonyl]-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^h 1-[[[1-(R)-1-[3-[(1S)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfonyl]-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

ⁱ 1-[[[1-(R)-1-[3-[(E)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-methylethenyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other individual degradation product	—	1.0	0.2
Total impurities	—	—	1.0

^a These two impurities are not resolved by the method and need to be integrated together to determine conformance.

^b 1-[[[1-(3-[(E)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl)-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^c (E)-1-[3-[2-(7-Chloroquinolin-2-yl)vinyl]phenyl]-3-[2-(2-hydroxypropan-2-yl)phenyl]propan-1-one.

^d 1-[[[1-(R)-1-[3-[(Z)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^e This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.

^f 1-[[[1-(R)-3-(2-Acetylphenyl)-1-[3-[(E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^g 1-[[[1-(R)-1-[3-[(1R)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfonyl]-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

^h 1-[[[1-(R)-1-[3-[(1S)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfonyl]-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

ⁱ 1-[[[1-(R)-1-[3-[(E)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-methylethenyl)phenyl]propyl]sulfonyl]methyl]cyclopropyl]acetic acid.

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
- LABELING** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- USP REFERENCE STANDARDS (11)**
USP Montelukast Dicyclohexylamine RS
 $C_{35}H_{36}ClNO_3S \cdot C_{12}H_{23}N$ 767.50

Montelukast Sodium Tablets

DEFINITION

Montelukast Sodium Tablets contain Montelukast Sodium equivalent to NLT 92.5% and NMT 107.5% of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$).

[NOTE—Avoid exposure of samples containing montelukast to light.]

IDENTIFICATION

A. ULTRAVIOLET ABSORPTION (197U)

Diluent: Methanol and water (3:1)

Standard solution: 0.026 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*

Sample solution: Nominally 0.02 mg/mL of montelukast prepared as follows. Transfer one Tablet equivalent to 10 mg of montelukast to a suitable volumetric flask, add 25% of the flask volume of water, and let stand for 5–10 min until the Tablet has disintegrated. Add 60% of the flask volume of methanol, shake well, and sonicate for 70 min with occasional shaking. Cool to room temperature, dilute with methanol to volume, and mix well. Centrifuge a portion of the resulting solution to obtain a clear solution.

Wavelength range: 210–400 nm

Acceptance criteria: The *Sample solution* exhibits maxima only at the same wavelengths as the *Standard solution*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Diluent: Methanol and water (3:1)

Solution A: 0.2% (v/v) Trifluoroacetic acid in water

Solution B: Methanol and acetonitrile (3:2)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	48	52
5	45	55
12	45	55
22	25	75
23	25	75
25	48	52
30	48	52

Standard solution: 0.52 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*

System suitability solution: Transfer 10 mL of the *Standard solution* to a clear 10-mL volumetric flask, add 4 μ L of hydrogen peroxide, and mix well. Expose the flask for at least 4 h to ambient light or 10 min to a 4 klx cool white light. [NOTE—Montelukast is partially converted to the *cis*-isomer under these conditions.]

Sensitivity solution: 0.52 μ g/mL of USP Montelukast Dicyclohexylamine RS in *Diluent* from the *Standard solution*

Sample solution: Nominally 0.4 mg/mL of montelukast prepared as follows. Transfer a number of Tablets equivalent to 100 mg of montelukast to a suitable volumetric flask, add 70% of the flask volume of *Diluent*, and sonicate for 30 min. Shake for 30 min on a platform shaker. Dilute with *Diluent* to volume and stir for 30 min. Pass a portion through a suitable filter of 0.45- μ m pore size, discarding the first mL of filtrate. Use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 255 nm

Columns

Guard: 3.0-mm \times 4-mm; packing L11

Analytical: 4.6-mm \times 10-cm; 3- μ m packing L11

Column temperature: 50°

Flow rate: 1.5 mL/min

Injection volume: 15 μ L

Run time: 2 times the retention time of montelukast

System suitability

Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

[NOTE—The relative retention times for the *cis*-isomer and montelukast are about 0.92 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between the *cis*-isomer and montelukast, *System suitability solution*

Relative standard deviation: NMT 2% for five injections, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of montelukast, 586.18

M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: 92.5%–107.5%

PERFORMANCE TESTS• **DISSOLUTION (711)****Test 1**

Medium: 0.5% (w/v) Sodium dodecyl sulfate in water; 900 mL. Do not deaerate.

Apparatus 2: 50 rpm

Time: 20 min

Solution A: 0.2% (v/v) Trifluoroacetic acid in water

Solution B: 0.2% (v/v) Trifluoroacetic acid in acetonitrile

Mobile phase: *Solution A* and *Solution B* (1:1)

Standard stock solution: 0.35 mg/mL of USP

Montelukast Dicyclohexylamine RS in methanol (equivalent to 0.27 mg/mL of montelukast)

Standard solution: (1/900) mg/mL of montelukast in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet of montelukast

Sample solution: Pass a portion of the solution under test through a suitable filter or centrifuge to obtain a clear solution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 389 nm

Column: 3.0-mm \times 10-cm; 5- μ m packing L11

Column temperature: 50°

Flow rate: 0.9 mL/min

Injection volume: 20 μ L

Run time: 1.5 times the retention time of montelukast

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of montelukast in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.5% (w/v) Sodium dodecyl sulfate in water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Solution A: 0.07 g/L of monobasic sodium phosphate

Solution B: Acetonitrile

Mobile phase: *Solution A* and *Solution B* (45:55). Add 1.33 mL/L of triethylamine and adjust with phosphoric acid to a pH of 6.7.

Standard stock solution: 0.1 mg/mL of montelukast from montelukast sodium hydrate prepared as follows. Transfer a suitable amount of montelukast sodium hydrate to a suitable volumetric flask. Dissolve in 5% of

the flask volume of methanol and dilute with *Medium* to volume. Determine the water content of montelukast sodium hydrate at the time of use.

Standard solution: 0.01 mg/mL of montelukast in *Medium* from the *Standard stock solution*

Sample solution: Centrifuge a portion of the solution under test.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 5-cm; 1.8-μm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 100 μL

Run time: 1.5 times the retention time of montelukast

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of montelukast in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 0.5% (w/v) Sodium dodecyl sulfate in water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Solution A: Acetonitrile and water (80:20)

Solution B: 3% Trifluoroacetic acid in *Solution A* prepared as follows. Transfer 3 mL of trifluoroacetic acid to a 100-mL volumetric flask and dilute with *Solution A* to volume.

Mobile phase: Acetonitrile, water, and *Solution B* (75:25:0.05).

Standard solution: ($L/900$) mg/mL of montelukast in *Medium* from montelukast sodium hydrate, where L is the label claim in mg/Tablet of montelukast. Determine the water content of montelukast sodium hydrate at the time of use.

Sample solution: Pass a portion of the solution under test through a suitable filter to obtain a clear solution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 346 nm

Column: 4.6-mm × 10-cm; 3-μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 25 μL

Run time: NLT 1.5 times the retention time of montelukast

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of montelukast in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Solution A, Solution B, Mobile phase, and System suitability: Proceed as directed in *Dissolution Test 1*.

Diluent: Methanol and water (3:1)

Standard solution: 0.052 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*

Sample solution: Nominally 0.04 mg/mL of montelukast prepared as follows. Transfer one Tablet equivalent to 10 mg of montelukast to a suitable volumetric flask, add 25% of the flask volume of water, and let stand for 5–10 min until the Tablet has disintegrated. Add 60% of the flask volume of methanol, shake well, and sonicate for 70 min with occasional shaking. Cool to room temperature, dilute with methanol to volume, and mix well. Pass a portion of the resulting solution through a suitable filter or centrifuge to obtain a clear solution.

Chromatographic system: Proceed as directed in *Dissolution Test 1*, except use an *Injection volume* of 10 μL.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of montelukast, 586.18

M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Diluent, Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, Sensitivity solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*

r_S = peak response of montelukast from the *Standard solution*

C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of montelukast, 586.18

M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

F = relative response factor (see *Table 2*)

Acceptance criteria: See Table 2. Disregard any peak with an area less than that of the *Sensitivity solution*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Sulfoxide impurity ^{a,b}	0.45	1.0	2.0
Montelukast ketone impurity ^c	0.71	1.7	0.2
<i>cis</i> -Isomer ^d	0.92	1.0	0.2
Montelukast	1.0	—	—
Methylketone impurity ^{e,f}	1.04	—	—
Michael adduct 1 ^{g,h}	1.16	—	—
Michael adduct 2 ^{h,i}	1.18	—	—
Methylstyrene impurity ^{j,k}	1.55	—	—
Any other individual degradation product	—	1.0	0.2
Total impurities	—	—	3.0

^a These two impurities are not resolved by the method and need to be integrated together to determine conformance.

^b 1-[[[1-[(3-[(2-(7-Chloroquinolin-2-yl)ethenyl]phenyl)-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl)sulfanyl]methyl]cyclopropyl]acetic acid.

^c (E)-1-[(3-[2-(7-Chloroquinolin-2-yl)vinyl]phenyl)-3-[2-(2-hydroxypropan-2-yl)phenyl]propan-1-one.

^d 1-[[[1-(R)-1-[(3-[(Z)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl)-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl)sulfanyl]methyl]cyclopropyl]acetic acid.

^e This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.

^f 1-[[[1-(R)-3-(2-Acetylphenyl)-1-[(3-[(E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl)propyl)sulfanyl]methyl]cyclopropyl]acetic acid.

^g 1-[[[1-(R)-1-[(3-[(1R)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfanyl]-2-(7-chloroquinolin-2-yl)ethyl]phenyl)-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl)sulfanyl]methyl]cyclopropyl]acetic acid.

^h 1-[[[1-(R)-1-[(3-[(1S)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfanyl]-2-(7-chloroquinolin-2-yl)ethyl]phenyl)-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl)sulfanyl]methyl]cyclopropyl]acetic acid.

ⁱ 1-[[[1-(R)-1-[(3-[(E)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl)-3-[2-(1-methylethyl)phenyl]propyl)sulfanyl]methyl]cyclopropyl]acetic acid.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
- **LABELING** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
USP Montelukast Dicyclohexylamine RS
 $C_{35}H_{36}ClNO_3S \cdot C_{12}H_{23}N$ 767.50

Montelukast Sodium Chewable Tablets

DEFINITION

Montelukast Sodium Chewable Tablets contain Montelukast Sodium equivalent to NLT 92.5% and NMT 107.5% of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$).

[NOTE—Avoid exposure of samples containing montelukast to light.]

IDENTIFICATION

• A. ULTRAVIOLET ABSORPTION (197U)

Diluent: Methanol and water (3:1)

Standard solution (for 4-mg Chewable Tablets):

0.026 mg/mL of USP Montelukast Dicyclohexylamine RS in Diluent

Standard solution (for 5-mg Chewable Tablets):

0.033 mg/mL of USP Montelukast Dicyclohexylamine RS in Diluent

Sample solution: Nominally (L/200) mg/mL of montelukast, where L is the label claim of montelukast in mg/Chewable Tablet prepared as follows. Transfer 1 Chewable Tablet to a suitable volumetric flask, add 25% of the flask volume of water, and let stand for 5–10 min until the Chewable Tablet has disintegrated. Add 55% of the flask volume of methanol, shake well, and sonicate for 70 min with occasional shaking. Cool to room temperature, dilute with methanol to volume, and mix well. Centrifuge a portion of the resulting solution to obtain a clear solution.

Wavelength range: 210–400 nm

Acceptance criteria: The *Sample solution* exhibits maxima only at the same wavelengths as the *Standard solution*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Diluent: Methanol and water (3:1)

Solution A: 0.2% (v/v) Trifluoroacetic acid in water

Solution B: Methanol and acetonitrile (3:2)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	48	52
5	45	55
12	45	55
22	25	75
23	25	75
25	48	52
30	48	52

Standard solution: 0.33 mg/mL of USP Montelukast Dicyclohexylamine RS in Diluent

System suitability solution: Transfer 10 mL of the *Standard solution* to a clear 10-mL volumetric flask, add 4 µL of hydrogen peroxide, and mix well. Expose the flask for at least 4 h to ambient light or 10 min to a 4 klx cool white light. [NOTE—Montelukast is partially converted to the *cis*-isomer under these conditions.]

Sensitivity solution: 0.33 µg/mL of USP Montelukast Dicyclohexylamine RS in Diluent from the *Standard solution*

Sample solution (for 4-mg Chewable Tablets): Nominally 0.24 mg/mL of montelukast prepared as follows. Transfer 12 Chewable Tablets to a suitable volumetric flask, add 75% of the flask volume of Diluent, and shake vigorously for 60 min. Dilute with Diluent to volume. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size, discarding the first mL of filtrate. Use the filtrate.

Sample solution (for 5-mg Chewable Tablets): Nominally 0.25 mg/mL of montelukast prepared as follows. Transfer 10 Chewable Tablets to a suitable volumetric flask, add 75% of the flask volume of Diluent, and shake vigorously for 60 min. Dilute with Diluent to volume. Pass a portion of the resulting solution through a suitable filter of 0.45-µm pore size, discarding the first mL of filtrate. Use the filtrate.

Chromatographic system(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 255 nm**Columns****Guard:** 3.0-mm × 4-mm; packing L11**Analytical:** 4.6-mm × 10-cm; 3-μm packing L11**Column temperature:** 50°**Flow rate:** 1.5 mL/min**Injection volume:** 20 μL**Run time:** 2 times the retention time of montelukast**System suitability****Samples:** *Standard solution*, *System suitability solution*, and *Sensitivity solution*[NOTE—The relative retention times for the *cis*-isomer and montelukast are about 0.92 and 1.0, respectively.]**Suitability requirements****Resolution:** NLT 1.5 between the *cis*-isomer and montelukast, *System suitability solution***Relative standard deviation:** NMT 2% for five injections, *Standard solution***Signal-to-noise ratio:** NLT 10, *Sensitivity solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) in the portion of Chewable Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL) C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL) M_{r1} = molecular weight of montelukast, 586.18 M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50**Acceptance criteria:** 92.5%–107.5%**PERFORMANCE TESTS****• DISSOLUTION <711>****Test 1****Medium:** 0.5% (w/v) Sodium dodecyl sulfate in water; 900 mL. Do not deaerate.**Apparatus 2:** 50 rpm**Time:** 20 min**Solution A:** 0.2% (v/v) Trifluoroacetic acid in water**Solution B:** 0.2% (v/v) Trifluoroacetic acid in acetonitrile**Mobile phase:** *Solution A* and *Solution B* (1:1)**Standard stock solution (for 4-mg Chewable Tablets):** 0.30 mg/mL of USP Montelukast Dicyclohexylamine RS in methanol (equivalent to 0.23 mg/mL of montelukast)**Standard stock solution (for 5-mg Chewable Tablets):** 0.35 mg/mL of USP Montelukast Dicyclohexylamine RS in methanol (equivalent to 0.27 mg/mL of montelukast)**Standard solution:** ($L/900$) mg/mL of montelukast in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Chewable Tablet of montelukast**Sample solution:** Pass a portion of the solution under test through a suitable filter or centrifuge to obtain a clear solution.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 389 nm**Column:** 3.0-mm × 10-cm; 5-μm packing L11**Column temperature:** 50°**Flow rate:** 0.9 mL/min**Injection volume:** 50 μL**Run time:** 1.5 times the retention time of montelukast**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 2%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of montelukast in the *Standard solution* (mg/mL) V = volume of *Medium*, 900 mL L = label claim (mg/Chewable Tablet)**Tolerances:** NLT 80% (Q) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.**Medium:** 0.5% (w/v) Sodium dodecyl sulfate in water; 900 mL**Apparatus 2:** 50 rpm**Time:** 45 min**Solution A:** 0.07 g/L of monobasic sodium phosphate**Solution B:** Acetonitrile**Mobile phase:** *Solution A* and *Solution B* (45:55). Add 1.33 mL/L of triethylamine and adjust with phosphoric acid to a pH of 6.7.**Standard stock solution:** 0.1 mg/mL of montelukast from montelukast sodium hydrate prepared as follows. Transfer a suitable amount of montelukast sodium hydrate to an appropriate volumetric flask. Dissolve in 4% of the flask volume of methanol and dilute with *Medium* to volume. Determine the water content of montelukast sodium hydrate at the time of use.**Standard solution:** 0.005 mg/mL of montelukast in *Medium* from the *Standard stock solution***Sample solution:** Centrifuge a portion of the solution under test.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 225 nm**Column:** 4.6-mm × 5-cm; 1.8-μm packing L1**Column temperature:** 35°**Flow rate:** 1 mL/min**Injection volume:** 100 μL**Run time:** 1.5 times the retention time of montelukast**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution*

C_S = concentration of montelukast in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Chewable Tablet)

Tolerances: NLT 70% (Q) of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Solution A, Solution B, Mobile phase, and System suitability: Proceed as directed in *Dissolution Test 1*.

Diluent: Methanol and water (3:1)

Standard solution (for 4-mg Chewable Tablets):

0.026 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*

Standard solution (for 5-mg Chewable Tablets):

0.033 mg/mL of USP Montelukast Dicyclohexylamine RS in *Diluent*

Sample solution: Nominally ($L/200$) mg/mL of montelukast, where L is the label claim of montelukast in mg/Chewable Tablet prepared as follows. Transfer 1 Chewable Tablet to a suitable volumetric flask, add 25% of the flask volume of water, and let stand for 5–10 min until the Chewable Tablet has disintegrated. Add 55% of the flask volume of methanol, shake well, and sonicate for 70 min with occasional shaking. Cool to room temperature, dilute with methanol to volume, and mix well. Pass a portion of the resulting solution through a suitable filter or centrifuge to obtain a clear solution.

Chromatographic system: Proceed as directed in *Dissolution Test 1*, except use an *Injection volume* of 10 μ L.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$) in the Chewable Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of montelukast, 586.18

M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

Acceptance criteria: Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Diluent, Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, Sensitivity solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Chewable Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of any individual degradation product from the *Sample solution*

r_S = peak response of montelukast from the *Standard solution*

C_S = concentration of USP Montelukast Dicyclohexylamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of montelukast in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of montelukast, 586.18

M_{r2} = molecular weight of montelukast dicyclohexylamine, 767.50

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard any peak with an area less than that of the *Sensitivity solution*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Sulfoxide impurity ^{a,b}	0.45	1.0	1.5
Montelukast ketone impurity ^c	0.71	1.7	0.2
<i>cis</i> -Isomer ^d	0.92	1.0	0.2
Montelukast	1.0	—	—
Methylketone impurity ^{e,f}	1.04	—	—
Michael adduct 1 ^{g,h}	1.16	—	—
Michael adduct 2 ^{h,i}	1.18	—	—
Methylstyrene impurity ^{j,k}	1.55	—	—
Any other individual degradation product	—	1.0	0.2
Total impurities	—	—	2.0

^a These two impurities are not resolved by the method and need to be integrated together to determine conformance.

^b 1-[[[1-(3-[(E)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl)-3-(2-(1-hydroxy-1-methylethyl)phenyl)propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

^c (E)-1-[3-(2-(7-Chloroquinolin-2-yl)vinyl)phenyl]-3-[2-(2-hydroxypropan-2-yl)phenyl]propan-1-one.

^d 1-[[[1-(R)-1-[3-[(Z)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-(2-(1-hydroxy-1-methylethyl)phenyl)propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

^e This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.

^f 1-[[[1-(R)-3-(2-Acetylphenyl)-1-[3-[(E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

^g 1-[[[1-(R)-1-[3-[(1R)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfanyl]-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-(2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

^h 1-[[[1-(R)-1-[3-[(1S)-1-[[[1-(Carboxymethyl)cyclopropyl]methyl]sulfanyl]-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-(2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

ⁱ 1-[[[1-(R)-1-[3-[(E)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl]-3-(2-(1-methylethyl)phenyl)propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

ADDITIONAL REQUIREMENTS

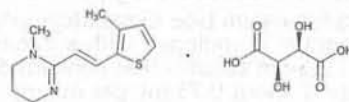
• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.

• **LABELING** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

• USP REFERENCE STANDARDS (11)

USP Montelukast Dicyclohexylamine RS
 $C_{35}H_{36}ClNO_3S \cdot C_{12}H_{23}N$ 767.50

Morantel Tartrate



$C_{12}H_{16}N_2S \cdot C_4H_6O_6$ 370.42

Pyrimidine, 1,4,5,6-tetrahydro-1-methyl-2-[2-(3-methyl-2-thienyl)ethenyl]-, (*E*)-, [*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1).
 (*E*)-1,4,5,6-Tetrahydro-1-methyl-2-[2-(3-methyl-2-thienyl)vinyl]pyrimidine tartrate (1:1) [26155-31-7].
 Morantel [20574-50-9].

» Morantel Tartrate contains not less than 96.4 percent and not more than 101.5 percent of $C_{12}H_{16}N_2S \cdot C_4H_6O_6$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

Labeling—Label it to indicate it is for veterinary use only.

USP Reference standards (11)—

USP Morantel Tartrate RS

Clarity and color of solution—Dissolve and dilute 0.25 g to 25.0 mL in carbon dioxide-free water. The solution is clear and yellow to greenish yellow in color.

Identification—

A: Infrared Absorption (197K).

B: It meets the requirements of the test for *Tartrate* (191).

C: The retention time of the morantel peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of *Standard solution 1*, as obtained in the test for *Related compounds*.

Melting temperature (741): 167° to 172°.

pH (791): between 2.8 and 3.9.

Solution—Dissolve and dilute 0.25 g to 25.0 mL in carbon dioxide-free water.

Loss on drying (731)—Dry it at 100° to 105° to constant weight: it loses not more than 1.5% of its weight.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals, Method II** (231)—not more than 20 ppm.

• (Official 1-Jan-2018)

Related compounds—[NOTE—Conduct this test without exposure to daylight, and with the minimum necessary exposure to artificial light.]

Mobile phase—Mix 3.5 mL of triethylamine and 850 mL of water. Adjust with phosphoric acid to a pH of 2.5. Add 50 mL of tetrahydrofuran and 100 mL of methanol, and mix.

Tartrate solution—Prepare a solution containing about 0.15 mg of tartaric acid per mL in *Mobile phase*.

Standard solution 1—Dissolve an accurately weighed quantity of USP Morantel Tartrate RS in *Mobile phase* to obtain a solution having a known concentration of about 5.0 µg per mL.

Standard solution 2—Dilute 2.0 mL of *Standard solution 1* to 100.0 mL with *Mobile phase*.

System suitability solution—Expose 10 mL of *Standard solution 1* to daylight for 15 minutes before injection.

Test solution—Dissolve an accurately weighed quantity of Morantel Tartrate in *Mobile phase* to obtain a solution having a concentration of about 0.5 mg per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 226-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 0.75 mL per minute. Chromatograph the *Tartrate solution*, *Standard solution 1*, and the *System suitability solution*, and record the peak areas as directed for *Procedure*: using the *System suitability solution*, the resolution, *R*, between morantel and its preceding peak ((*Z*)-iso-

mer) is not less than 2. The relative retention times are about 0.8, 1.0, and 1.2 for the morantel (*Z*)-isomer, morantel, and the morantel 4-methyl isomer (1-methyl-2-[(*E*)-2-(4-methylthiophen-2-yl)ethenyl]-1,4,5,6-tetrahydropyrimidine), respectively.

Procedure—Separately inject equal volumes (about 20 µL) of the *Tartrate solution*, *Standard solution 1*, *Standard solution 2*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Disregarding the tartrate peak and any peak in the chromatogram of the *Test solution* less than the area of the principal peak in the chromatogram of *Standard solution 2*, calculate the area percentage of each impurity, relative to morantel, in the portion of Morantel Tartrate taken by the formula:

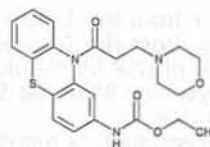
$$100(C_S / C_U)(r_i / r_S)$$

in which C_S and C_U are the concentrations of morantel tartrate, in mg per mL, of *Standard solution 1* and the *Test solution*, respectively; and r_i and r_S are the peak areas of each individual impurity and morantel obtained from the *Test solution* and *Standard solution 1*, respectively: not more than 3% of the morantel 4-methyl isomer is found; not more than 0.5% of any other individual impurity is found; and not more than 1% of total other individual impurities is found.

Assay—

Dissolve 0.280 g in 40 mL of anhydrous acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically (see *Titrimetry* (541)). One mL of 0.1 N perchloric acid is equivalent to 37.04 mg of $C_{12}H_{16}N_2S \cdot C_4H_6O_6$.

Moricizine Hydrochloride



$C_{22}H_{25}N_3O_4S \cdot HCl$ 463.98
 Carbamic acid, [10-[3-(4-morpholinyl)-1-oxopropyl]-10*H*-phenothiazin-2-yl]-, ethyl ester, hydrochloride;
 Ethyl 10-(3-morpholinopropionyl)phenothiazine-2-carbamate, hydrochloride [29560-58-5].

DEFINITION

Moricizine Hydrochloride contains NLT 98.0% and NMT 102.0% of moricizine hydrochloride ($C_{22}H_{25}N_3O_4S \cdot HCl$), calculated on the anhydrous and alcohol-free basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements

ASSAY

- **PROCEDURE:** Use low-actinic glassware. Protect the solutions containing moricizine hydrochloride from light.
Mobile phase: A mixture of acetonitrile, triethylamine, glacial acetic acid, and water (420:1:20:580) containing 5 mM sodium 1-octane sulfonate
Diluent: Acetonitrile and 0.02 N hydrochloric acid (42:58)
Internal standard solution: 5 mg/mL of butamben in *Diluent*

Standard solution: 1 mg/mL of USP Moricizine Hydrochloride RS in *Diluent* prepared as follows. Transfer a suitable amount of USP Moricizine Hydrochloride RS to a suitable volumetric flask, add *Internal standard solution* to fill about 20% of the total volume, and dilute with *Diluent* to volume.

Sample solution: 1 mg/mL of Moricizine Hydrochloride prepared as follows. To a suitable amount of Moricizine Hydrochloride in a 50-mL volumetric flask add *Internal standard solution* to fill about 20% of the total volume, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 35°

Flow rate: 2.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for moricizine, butamben, the reverse Mannich product, and the amide hydrolysis product are about 0.6, 1.0, 1.7, and 2.0, respectively.]

Suitability requirements

Resolution: NLT 2 between moricizine and butamben

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of moricizine hydrochloride ($C_{22}H_{25}N_3O_4S \cdot HCl$) in the portion of Moricizine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the moricizine peak area response to the butamben peak area response from the *Sample solution*

R_S = ratio of the moricizine peak area response to the butamben peak area response from the *Standard solution*

C_S = concentration of USP Moricizine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Moricizine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and alcohol-free basis

OTHER COMPONENTS

• CONTENT OF CHLORIDE

Sample solution: Transfer 400 mg of Moricizine Hydrochloride to a conical flask and add 75 mL of methanol. Swirl to dissolve. Add 5 mL of glacial acetic acid and 3 drops of eosin Y TS.

Analysis: Titrate the *Sample solution* with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.546 mg of chloride.

Acceptance criteria: 7.49%–7.80% on the anhydrous and alcohol-free basis

IMPURITIES

• RESIDUE ON IGNITION (281): NMT 0.1%

Delete the following:

• HEAVY METALS, Method II (231): NMT 10 µg/g (Official 1-

Jan-2018)

• ORGANIC IMPURITIES

Mobile phase: A mixture of acetonitrile, triethylamine, and water (420:1:580) containing 5 mM sodium 1-oc-

tane sulfonate. Adjust with glacial acetic acid to a pH of 4.2.

Diluent: Acetonitrile and 0.02 N hydrochloric acid (42:58)

Internal standard solution: 0.1 mg/mL of butamben in *Diluent*

Standard stock solution: 0.10 mg/mL of USP Moricizine Hydrochloride RS in *Diluent*

Standard solution: 2.0 µg/mL of USP Moricizine Hydrochloride RS prepared as follows. Transfer a suitable volume of *Standard stock solution* to a suitable volumetric flask, add *Internal standard solution* to fill 5% of the total volume, and dilute with *Diluent* to volume.

[NOTE—Protect the solution from light.]

Sample solution: 1 mg/mL of Moricizine Hydrochloride in *Diluent* prepared as follows. Transfer a suitable amount of Moricizine Hydrochloride to a suitable volumetric flask, add *Internal standard solution* to fill 5% of the total volume, and dilute with *Diluent* to volume.

[NOTE—Protect the solution from light.]

Chromatographic system: Proceed as directed in the *Assay* except for the following:

Run time: Five times the elution time of moricizine

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for moricizine and butamben are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2 between moricizine and butamben

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the responses for all the peaks except the solvent peak.

Calculate the percentage of each impurity in the portion of Moricizine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the peak area response of each impurity to the butamben peak area response from the *Sample solution*

R_S = ratio of the peak area response of moricizine to the peak area response of butamben from the *Standard solution*

C_S = concentration of USP Moricizine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: Disregard any impurity of less than 0.1%.

Any impurity eluting before moricizine: NMT 0.25%

Any impurity eluting after moricizine: NMT 0.20%

Total of all impurities: NMT 1.5%

• LIMIT OF ALCOHOL

Standard solution: 0.1184 mg/mL of dehydrated alcohol in water prepared as follows. Transfer 6.0 mL of dehydrated alcohol to a 100-mL volumetric flask and dilute with water to volume. Dilute 5.0 mL of this solution in a 100-mL volumetric flask with water to volume. Further dilute 5.0 mL of this solution in a third 100-mL volumetric flask with water to volume.

Sample solution: Transfer 1 g of Moricizine Hydrochloride to a 50-mL glass-stoppered centrifuge tube, add 19.0 mL of water, and sonicate to dissolve. Transfer 1.0 mL of 3 N ammonium hydroxide to the tube, insert the stopper, and shake the tube by mechanical means for 30 min. Centrifuge, draw off a portion of the clear supernatant, and pass through a suitable filter of 0.5-µm or finer pore size.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** GC**Detector:** Flame ionization**Column:** 4-mm × 1.8-m glass; supporting S2**Temperatures****Column:** 150°**Injector port:** 170°**Detector block:** 170°**Carrier gas:** Helium**Flow rate:** 50 mL/min**Injection volume:** 5 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 3%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of alcohol (C₂H₅OH) in the portion of Moricizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of alcohol from the *Sample solution* r_S = peak response of alcohol from the *Standard solution* C_S = concentration of alcohol in the *Standard solution* (mg/mL) C_U = concentration of Moricizine Hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria:** NMT 0.25%**SPECIFIC TESTS**• **LOSS ON DRYING** (731)**Analysis:** Dry at 105° for 4 h.**Acceptance criteria:** NMT 1.0%• **WATER DETERMINATION, Method I** (921): NMT 1.0%• **CLARITY OF SOLUTION****Sample solution:** Dissolve 1 g in 30 mL of methanol, sonicating for 5 min if necessary.**Acceptance criteria:** The solution is not less clear than an equal volume of methanol contained in a similar vessel and examined similarly.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight containers.• **USP REFERENCE STANDARDS** (11)

USP Moricizine Hydrochloride RS

Moricizine Hydrochloride Tablets

» Moricizine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of moricizine hydrochloride (C₂₂H₂₅N₃O₄S · HCl).

Packaging and storage—Preserve in tight containers.**USP Reference standards** (11)—

USP Moricizine Hydrochloride RS

Identification—**A: Ultraviolet Absorption** (197U)—

Test solution—Transfer a portion of finely ground Tablets, equivalent to about 50 mg of moricizine hydrochloride, to a 250-mL volumetric flask, add about 100 mL of 0.1 N hydrochloric acid, shake by mechanical means for 15 minutes, dilute with 0.1 N hydrochloric acid to volume, and mix. Filter a portion of this solution, discarding the first 10 mL of the filtrate. Transfer 10 mL of the filtrate to a 250-mL volumetric

flask, dilute with 0.1 N hydrochloric acid to volume, and mix.

Standard solution: 8 µg per mL.**Medium:** 0.1 N hydrochloric acid.

B: Shake a Tablet with 10 mL of methanol until it disintegrates, and filter: the filtrate responds to *Identification test C* under *Moricizine Hydrochloride*.

Dissolution (711)—**Medium:** 0.1 N hydrochloric acid; 900 mL.**Apparatus 2:** 50 rpm.**Time:** 30 minutes.

Procedure—Determine the amount of moricizine hydrochloride (C₂₂H₂₅N₃O₄S · HCl) dissolved from UV absorbance at about 267 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a *Standard solution* having a known concentration of USP Moricizine Hydrochloride RS in the same medium.

Tolerances—Not less than 75% (Q) of the labeled amount of moricizine hydrochloride (C₂₂H₂₅N₃O₄S · HCl) is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.**Limit of degradation products**—

Mobile phase—Dissolve 1.08 g of sodium 1-octanesulfonate in 580 mL of water, add 420 mL of acetonitrile, 20 mL of glacial acetic acid, and 1 mL of triethylamine. Adjust with 5 N sodium hydroxide to an apparent pH of 4.5. Mix, and filter through a filter having a porosity of 0.5 µm or finer. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Diluent—Prepare a mixture of 0.02 N hydrochloric acid and acetonitrile (58:42).

Internal standard solution—Prepare a solution of butamben in *Diluent* containing about 0.2 mg per mL.

Standard solution—Prepare a solution of USP Moricizine Hydrochloride RS in *Diluent* containing 0.10 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Diluent* to volume, and mix. [NOTE—Protect this solution from light.]

Test solution—Transfer 10 Tablets to a 1000-mL flask, add 500.0 mL of *Diluent*, sonicate until the Tablets are disintegrated, and then shake by mechanical means for 30 minutes. Filter this solution, discarding the first 10 mL of the filtrate. Transfer 25.0 mL of the filtrate to a 50-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Diluent* to volume, and mix. [NOTE—Protect this solution from light.]

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7 and is maintained at a constant temperature of about 35°. The flow rate is about 2.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.6 for moricizine and 1.0 for butamben, the resolution, R , between the moricizine peak and the butamben peak is not less than 2, and the relative standard deviation for replicate injections is not more than 5%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for a period of time that is five times the elution time of moricizine, and measure the responses for the peaks, except for any that elute before moricizine. Calculate the percentage of each impurity peak that elutes after butamben in the portion of Moricizine Hydrochloride taken by the formula:

$$100(C/L)(R_i/R_S)$$

in which C is the concentration, in mg per mL, of USP Moricizine Hydrochloride RS in the *Standard solution*, L is the

labeled amount, in mg, of moricizine hydrochloride in each Tablet, R_i is the ratio of the peak areas of an individual impurity peak to the butamben peak obtained from the *Test solution*, and R_s is the ratio of the peak areas of the moricizine peak to the butamben peak obtained from the *Standard solution*. The first impurity eluting after the butamben peak is not more than 0.50%, and the second impurity eluting after butamben is not more than 0.25%.

Assay—

Mobile phase, Diluent, Internal standard solution, Standard preparation, and Chromatographic system—Proceed as directed in the Assay under Moricizine Hydrochloride.

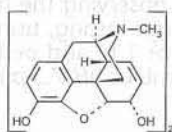
Assay preparation—Transfer an accurately counted number of Tablets, equivalent to about 4000 mg of moricizine hydrochloride, to a 2000-mL flask, add 1000.0 mL of *Diluent*, and sonicate until the Tablets have disintegrated. Shake by mechanical means for 30 minutes. Filter a portion of this solution, discarding the first 10 mL of the filtrate. Cover the filter funnel with a watch glass to minimize evaporation of the solvent. Transfer 25.0 mL of the filtrate and 20.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. [NOTE—Protect this solution from light.]

Procedure—Proceed as directed for *Procedure* in the Assay under Moricizine Hydrochloride. Calculate the quantity, in mg, of moricizine hydrochloride ($C_{22}H_{25}N_3O_4 \cdot HCl$) in each Tablet by the formula:

$$4000(C/N)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Moricizine Hydrochloride RS in the *Standard preparation*, N is the number of Tablets taken, and R_U and R_S are the ratios of the peak area responses of the moricizine peak to the butamben peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Morphine Sulfate



$(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ 758.83

Morphinan-3,6-diol, 7,8-didehydro-4,5-epoxy-17-methyl-, (5 α ,6 α)-, sulfate (2:1) (salt), pentahydrate.

7,8-Didehydro-4,5 α -epoxy-17-methylmorphinan-3,6 α -diol sulfate (2:1) (salt) pentahydrate [6211-15-0].

Anhydrous 668.77 [64-31-3].

» Morphine Sulfate contains not less than 98.0 percent and not more than 102.0 percent of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store up to 40° as permitted by the manufacturer.

USP Reference standards (11)—

USP Morphine Sulfate RS

Identification—

A: *Infrared Absorption* (197K): dried at 145° for 1 hour.

B: To 1 mg in a porcelain crucible or small dish add 0.5 mL of sulfuric acid containing, in each mL, 1 drop of formaldehyde TS: an intense purple color is produced at once, and quickly changes to deep blue-violet (*distinction from codeine, which gives at once an intense violet-blue color,*

and from hydromorphone, which gives at first a yellow to brown color, changing to pink and then to purplish red).

C: To a solution of 5 mg in 5 mL of sulfuric acid in a test tube add 1 drop of ferric chloride TS, mix, and heat in boiling water for 2 minutes: a blue color is produced, and when 1 drop of nitric acid is added, it changes to dark red-brown (*codeine and ethylmorphine give the same color reactions, but hydromorphone and papaverine do not produce this color change*).

D: A solution (1 in 50) responds to the tests for Sulfate (191).

Specific rotation (781S): between -107° and -109.5° .

Test solution: the equivalent of 20 mg per mL, in water.

Acidity—Dissolve 500 mg in 15 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 0.50 mL is required to produce a yellow color.

Water Determination, Method I (921): between 10.4% and 13.4% is found.

Residue on ignition (281): not more than 0.1%, from 500 mg.

Chloride—To 10 mL of a solution (1 in 100) add 1 mL of 2 N nitric acid and 1 mL of silver nitrate TS: no precipitate or turbidity is produced immediately.

Ammonium salts—Heat 200 mg with 5 mL of 1 N sodium hydroxide on a steam bath for 1 minute: no odor of ammonia is perceptible.

Limit of foreign alkaloids—Dissolve 1.00 g in 10 mL of 1 N sodium hydroxide in a separator, and shake the solution with three successive portions of 15, 10, and 10 mL of chloroform, passing the chloroform solutions through a small filter previously moistened with chloroform. Shake the combined chloroform solutions with 5 mL of water, separate the chloroform layer, and carefully evaporate on a steam bath to dryness. To the residue add 10.0 mL of 0.020 N sulfuric acid, and heat gently until dissolved. Cool, add 2 drops of methyl red TS, and titrate the excess acid with 0.020 N sodium hydroxide: not less than 7.5 mL is required (1.5%).

Assay—

Mobile phase—Dissolve 0.73 g of sodium 1-heptanesulfonate in 720 mL of water, add 280 mL of methanol and 10 mL of glacial acetic acid, mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Morphine Sulfate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.24 mg per mL. Prepare a fresh solution daily.

System suitability preparation—Dissolve suitable quantities of USP Morphine Sulfate RS and phenol in *Mobile phase* to obtain a solution containing about 0.24 and 0.15 mg per mL, respectively.

Assay preparation—Transfer about 24 mg of Morphine Sulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 284-nm detector and a 3.9-mm \times 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for phenol and 1.0 for morphine sulfate; the resolution, R , between phenol and morphine sulfate is not less than 2.0; the tailing factor for the morphine sulfate peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and meas-

ure the responses for the major peaks. Calculate the quantity, in mg, of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$ in the portion of Morphine Sulfate taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of anhydrous morphine sulfate in the *Standard preparation*, as determined from the concentration of USP Morphine Sulfate RS corrected for moisture content by a titrimetric water determination; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Morphine Sulfate Extended-Release Capsules

DEFINITION

Morphine Sulfate Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Diluent: Water. Adjust with phosphoric acid to a pH of 3.6.

Buffer solution: 13.8 mg/mL of monobasic sodium phosphate

Solution A: Acetonitrile, triethylamine, *Buffer solution*, and water (25:0.5:100:874.5). Adjust with phosphoric acid to a pH of 3.6.

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
33	100	0
44	85	15
54	85	15
55	100	0
65	100	0

System suitability solution: 400 µg/mL of USP Morphine Sulfate RS, 10 µg/mL of USP Morphine Related Compound A RS, and 10 µg/mL of USP Morphine Related Compound B RS (pseudomorphine) in *Diluent*

Standard solution: 1.0 mg/mL of USP Morphine Sulfate RS in *Diluent*

Sample stock solution: Transfer a weighed portion of the contents from NLT 20 Capsules, nominally equivalent to 250 mg of morphine sulfate pentahydrate, to a 100-mL volumetric flask. Add 5 mL of methanol, and mix well for 30 min with gentle swirling every 5 min. Add *Diluent* up to half of the flask volume, and sonicate for 5 min to dissolve. Dilute with *Diluent* to volume.

Sample solution: Nominally 1.0 mg/mL of morphine sulfate pentahydrate from *Sample stock solution* in *Diluent*. Pass through a suitable filter, and use the clear filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Columns

Guard: Packing L1

Analytical: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 40 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the morphine related compound A and morphine sulfate peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ in the portion of Capsules taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Morphine Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of morphine sulfate pentahydrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Test 1

pH 7.5 phosphate buffer: 6.8 mg/mL of monobasic potassium phosphate and 1.6 mg/mL of sodium hydroxide. Adjust with phosphoric acid or 2 N sodium hydroxide to a pH of 7.5.

Medium: Proceed as directed in *Dissolution* (711), *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms, Method B*, observing the following exceptions. Perform *Acid Stage* testing, using 500 mL of 0.1 N hydrochloric acid for 1 h; and perform *Buffer Stage* testing, using 500 mL of pH 7.5 phosphate buffer for NLT 8 h.

Apparatus 1: 100 rpm

Times: 1, 4, 6, and 9 h

Mobile phase: Methanol, glacial acetic acid, and water (280:10:720), containing 0.73 g of sodium 1-heptanesulfonate for each 1.01 L of the solvent mixture

System suitability solution: 0.1 mg/mL each of phenol and USP Morphine Sulfate RS in *Mobile phase*

Standard solution: USP Morphine Sulfate RS in pH 7.5 phosphate buffer to obtain a solution with a known concentration corresponding to that of the *Sample solution*

Sample solution: Sample per *Dissolution* (711).

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 284 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 25 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for phenol and morphine sulfate are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the phenol and morphine sulfate peaks

Tailing factor: NMT 2.0 for the morphine sulfate peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution and Sample solution*

Tolerances: See Table 2.

Table 2

Time (h)	Amount Dissolved (%)
1	NMT 10
4	25–50
6	50–90
9	NLT 85

The percentage of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ dissolved in 1 h conforms to *Dissolution* (711), *Extended-Release Dosage Forms, Acceptance Table 3*. The percentages of the labeled amount of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ dissolved at the other times specified conform to *Dissolution* (711), *Extended-Release Dosage Forms, Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium

Acid stage: 0.1 N hydrochloric acid (HCl); 500 mL

Buffer stage: pH 7.5 phosphate buffer (Dissolve 40.8 g of monobasic potassium phosphate and 9.6 g of sodium hydroxide in 6 L of water. Adjust with phosphoric acid or 2 N sodium hydroxide to a pH of 7.5.); 500 mL

Apparatus 1: 100 rpm

Times: 1, 4, 6, and 9 h

Solution A: 0.1% Phosphoric acid and 0.1% triethylamine in water

Mobile phase: *Solution A* and methanol (93:7)

Standard stock solution: 2.0 mg/mL of USP Morphine Sulfate RS in water

Standard solution: 0.16 mg/mL of USP Morphine Sulfate RS in either the *Acid stage* under *Medium* or in the *Buffer stage* under *Medium*, from *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter of 10- μ m pore size. Centrifuge the filtrate if necessary.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 25°

Flow rate: 1.5 mL/min

Injection volume: 5 μ L

Run time: NLT 2 times the retention time of morphine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution and Sample solution*

Replace the *Acid stage* under *Medium* immediately after 1 h with the *Buffer stage* under *Medium*.

Calculate the concentration (C_i) of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

r_U = peak response of morphine from the *Sample solution* at each time point (i)

r_S = peak response of morphine from the appropriate *Standard solution* at each time point (i)

C_S = concentration of USP Morphine Sulfate RS in the appropriate *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = C_2 \times V \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times (V - V_S)] + (C_2 \times V_S)\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times (V - 2 \times V_S)] + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ in the portion of the sample withdrawn at each time point (i) (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Capsule)

V_S = volume of *Medium* taken (mL)

Tolerances: See Table 3.

Table 3

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 10
2	4	10–35
3	6	50–70
4	9	NLT 80

The percentages of the labeled amount of morphine sulfate pentahydrate released at the times specified conform to *Dissolution* (711), *Extended-Release Dosage Forms, Acceptance Table 2*.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Diluent, Solution A, System suitability solution, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Sensitivity solution: 0.5 μ g/mL of USP Morphine Sulfate RS in *Diluent*

System suitability

Samples: *System suitability solution, Standard solution, and Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0 between the morphine related compound A and morphine sulfate peaks, *System suitability solution*

Sensitivity: Morphine peak is detectable, *Sensitivity solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Diluent and Sample solution*. [NOTE—Disregard the peaks corresponding to those obtained in the chromatogram of the *Diluent*.]

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

- r_U = peak response of each impurity from the *Sample solution*
 r_S = peak response of morphine sulfate from the *Standard solution*
 F = relative response factor (see Table 4)
 Acceptance criteria: See Table 4.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Morphine related compound A ^a	1.4	1.0	0.5
Morphine sulfate	1.0	—	—
Morphine related compound B ^b	2.3	2.1	0.5
Any unspecified impurity	—	—	0.2
Total impurities	—	—	1.5

^a 7,8-Didehydro-4,5 α -epoxy-17-methylmorphinan-3,6 α -diol, N-oxide.

^b 2,2'-Bimorphine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one test for *Dissolution* is given, the *Labeling* section states the test for *Dissolution* used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
 USP Morphine Sulfate RS
 USP Morphine Related Compound A RS
 7,8-Didehydro-4,5 α -epoxy-17-methylmorphinan-3,6 α -diol, N-oxide.
 $C_{17}H_{19}NO_4$ 301.34
 USP Morphine Related Compound B RS
 2,2'-Bimorphine.
 $C_{34}H_{36}N_2O_6$ 568.66

Morphine Sulfate Injection

DEFINITION

Morphine Sulfate Injection is a sterile solution of Morphine Sulfate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$. Injection intended for intramuscular or intravenous administration may contain sodium chloride as a tonicity-adjusting agent, and suitable antioxidants and antimicrobial agents. Injection intended for intrathecal or epidural use may contain sodium chloride as a tonicity-adjusting agent, but contains no other added substances.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Sulfate (191):** It meets the requirements of the barium chloride test.

ASSAY

PROCEDURE

Mobile phase: Dissolve 0.73 g of sodium 1-heptanesulfonate in 720 mL of water, and add 280 mL of methanol and 10 mL of glacial acetic acid.

System suitability solution: 0.24 mg/mL of USP Morphine Sulfate RS and 0.15 mg/mL of phenol in *Mobile phase*

Standard solution: 0.24 mg/mL of USP Morphine Sulfate RS (on the anhydrous basis) in *Mobile phase*.

[NOTE—Prepare a fresh solution daily.]

Sample solution: Nominally 0.24 mg/mL of morphine sulfate from the Injection in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 284 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 25 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for phenol and morphine are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between phenol and morphine sulfate, *System suitability solution*

Tailing factor: NMT 2.0 for the morphine sulfate peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ in each mL of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Morphine Sulfate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

M_{r1} = molecular weight of morphine sulfate pentahydrate, 758.83

M_{r2} = molecular weight of anhydrous morphine sulfate, 668.77

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **PH (791):** 2.5–6.5
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 17.0 USP Endotoxin Units/mg of morphine sulfate. If labeled for intrathecal use, it contains NMT 14.29 USP Endotoxin Units/mg of morphine sulfate.
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements under small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light. Preserve Injection labeled "Preservative-free" in single-dose containers.
- **LABELING:** It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. Label it also to state that the Injection is not to be used if its color is darker than pale yellow, if it is discolored in any other way, or if it contains a precipitate. Injection containing no antioxidant or antimicrobial agents prominently bears on its label the words "Preservative-free" and includes in its labeling its routes of administration and the statement that it is not to be heat-sterilized. Injection containing antioxidant or antimicrobial agents includes in its labeling its routes of administration and the statement that it is not for intrathecal or epidural use.

- **USP REFERENCE STANDARDS** (11)
 - USP Endotoxin RS
 - USP Morphine Sulfate RS

Morphine Sulfate Compounded Suppositories

DEFINITION

Morphine Sulfate Compounded Suppositories contain NLT 90.0% and NMT 110.0% of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$. Prepare Morphine Sulfate Compounded Suppositories in Fatty Acid Base or Polyethylene Glycol Base as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Morphine Sulfate	50 mg
Silica Gel	25 mg
Fatty Acid Base or Polyethylene Glycol Base, a sufficient quantity to make	1 suppository

Calibrate the actual molds with the *Base* that is used for preparing the Suppositories, and adjust the formula accordingly. Thoroughly mix the *Morphine Sulfate* and *Silica Gel* to obtain a uniform powder. Heat the *Base* slowly and evenly until melted. Slowly add the powder to the melted *Base* with stirring. Mix thoroughly, and pour into molds. Cool, trim, and wrap.

ASSAY

• SUPPOSITORIES IN FATTY ACID BASE

Mobile phase: Dissolve 5.5 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol and 10 mL of glacial acetic acid. Filter and degas.

System suitability solution: 0.24 mg/mL of USP Morphine Sulfate RS and 0.15 mg/mL of phenol in *Mobile phase*.

Standard solution: 0.5 mg/mL of USP Morphine Sulfate RS in *Mobile phase*. Prepare a fresh solution daily.

Sample solution: Transfer 1 Suppository to a 60-mL separator containing 20 mL of chloroform and 20 mL of 0.01 N hydrochloric acid, and shake to dissolve the Suppository. Transfer the chloroform layer to a 250-mL separator. Extract the aqueous layer with a second 20-mL portion of chloroform, and combine the chloroform extracts in the 250-mL separator. Wash the chloroform extracts with two additional 20-mL portions of 0.01 N hydrochloric acid, combine the aqueous layers in a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass through a filter of 0.45- μ m or finer pore size, discarding the first 4 mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 284 nm

Column: 4.6-mm \times 25-cm; packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for phenol and morphine sulfate are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between phenol and morphine sulfate, *System suitability solution*

Tailing factor: NMT 2.0 for the morphine sulfate peak

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ in the Suppository:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Morphine Sulfate RS in the *Standard solution* (mg/mL) (corrected for moisture content by titrimetric determination)

C_U = nominal concentration of morphine sulfate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of morphine sulfate pentahydrate, 758.83

M_{r2} = molecular weight of anhydrous morphine sulfate, 668.77

Acceptance criteria: 90.0%–110.0%

• SUPPOSITORIES IN POLYETHYLENE GLYCOL BASE

Mobile phase, System suitability solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay for Suppositories in Fatty Acid Base*.

Sample solution: Transfer 1 Suppository to a 100-mL volumetric flask, and add 70 mL of *Mobile phase*. Sonicate for 15 min to dissolve the Suppository, cool, dilute with *Mobile phase* to volume, and mix. Pass a 10-mL portion of the solution through a filter of 0.45- μ m or finer pore size, discarding the first 4 mL of filtrate.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of morphine sulfate pentahydrate $[(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O]$ in the Suppository:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Morphine Sulfate RS in the *Standard solution* (mg/mL) (corrected for moisture content by titrimetric determination)

C_U = nominal concentration of morphine sulfate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of morphine sulfate pentahydrate, 758.83

M_{r2} = molecular weight of anhydrous morphine sulfate, 668.77

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements for *Weight Variation*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight containers, and store in a refrigerator. Do not dispense or store Polyethylene Glycol Base Suppositories in polystyrene containers.
- **BEYOND-USE DATE:** NMT 90 days after the date on which they were compounded when stored in a refrigerator
- **LABELING:** Label Morphine Sulfate Compounded Suppositories to state whether they are in a Fatty Acid Base or in a Polyethylene Glycol Base. Label to indicate that they are for rectal use only. Label to state that they are to be stored in a refrigerator. Label it to state the *Beyond-Use Date*.

• **USP REFERENCE STANDARDS** (11)
USP Morphine Sulfate RS

Morrhuate Sodium Injection

» Morrhuate Sodium Injection is a sterile solution of the sodium salts of the fatty acids of Cod Liver Oil. It contains, in each mL, not less than 46.5 mg and not more than 53.5 mg of morrhuate sodium. A suitable antimicrobial agent, not to exceed 0.5 percent, and ethyl alcohol or benzyl alcohol, not to exceed 3.0 percent, may be added.

NOTE—Morrhuate Sodium Injection may show a separation of solid matter on standing. Do not use the material if such solid does not dissolve completely upon warming.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass. It may be packaged in 50-mL multiple-dose containers.

USP Reference standards (11)—
USP Endotoxin RS

Identification—Evaporate about 5 mL of the chloroform solution of the fatty acids obtained in the test for *Iodine value of the fatty acids* on a steam bath nearly to dryness, dissolve the residue in 1 mL of chloroform, and add 1 drop of sulfuric acid: a transient red color is produced, and it changes to brown-red.

Bacterial Endotoxins Test (85)—It contains not more than 1.4 USP Endotoxin Units per mg of morrhuate sodium.

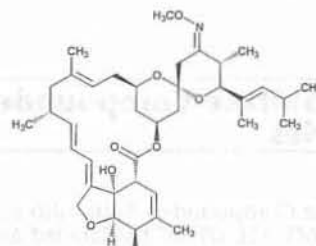
Acidity and alkalinity—To 5 mL of Injection add 5 mL of alcohol and 2 drops of phenolphthalein TS. If no red color is produced, not more than 0.50 mL of 0.10 N sodium hydroxide is required to impart a distinct red color. If a red color is produced, not more than 0.30 mL of 0.10 N acid is required to discharge it. For concentrations of morrhuate sodium other than 5%, no larger than proportional volumes of alkali and acid are required.

Iodine value of the fatty acids—Transfer to a tared, 125-mL conical flask the solvent hexane solution of the fatty acids obtained in the Assay. Evaporate at about 60° to dryness, dry the residue in vacuum over silica gel for 18 hours, and weigh. Dissolve the residue in chloroform to make 100.0 mL of solution, and determine the iodine value (see *Fats and Fixed Oils* (401)) on a 25.0-mL aliquot of the solution: the iodine value is not less than 130.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1), except that at times it may show a slight turbidity or precipitate.

Assay—Transfer an accurately measured volume of Injection, equivalent to about 500 mg of morrhuate sodium, to a small separator containing 30.0 mL of 0.1 N sulfuric acid VS, add 25 mL of solvent hexane, shake gently, and allow to separate. Withdraw the aqueous layer into a beaker or flask, and wash the solvent hexane layer with two 10-mL portions of water, adding the washings to the main aqueous solution. Retain the hexane solution for the test for *Iodine value of the fatty acids*. Add methyl orange TS, and titrate the excess acid in the aqueous solution with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sulfuric acid is equivalent to 32.4 mg of morrhuate sodium.

Moxidectin



$C_{37}H_{53}NO_8$ 639.82
(6*R*,25*S*)-5-*O*-Demethyl-28-deoxy-25-[(*E*)-1,3-dimethyl-1-butenyl]-6,28-epoxy-23-oxomilbemycin B 23-(*E*)-(*O*-methyloxime); (2*aE*,4*E*,5'*R*,6*R*,6'*S*,8*E*,11*R*,13*S*,15*S*,17*aR*,20*R*,20*aR*,20*bS*)-6'-[(*E*)-1,3-Dimethyl-1-butenyl]-5',6,6',7,10,11,14,15,17*a*,20,20*a*,20*b*-dodecahydro-20,20*b*-dihydroxy-5',6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2'-[2*H*]pyran]-4',17(3'*H*)-dione 4'-(*E*)-(O-methyloxime) [113507-06-5].

DEFINITION

Moxidectin contains NLT 92.0% and NMT 102.0% of moxidectin ($C_{37}H_{53}NO_8$), calculated on the anhydrous basis. It may contain a suitable antioxidant.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Buffer: Dissolve 7.7 g of ammonium acetate in 400 mL of water, and adjust with glacial acetic acid to a pH of 4.8.

Mobile phase: Acetonitrile and Buffer (60:40)

Standard solution: 1.0 mg/mL of USP Moxidectin RS in acetonitrile. Sonicate if necessary to facilitate dissolution.

Sample solution: 1.0 mg/mL of Moxidectin in acetonitrile. Sonicate if necessary to facilitate dissolution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 3.9-mm × 15-cm; 4-μm packing L1

Column temperature: 50°

Flow rate: 2.5 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 1%, for four replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of moxidectin ($C_{37}H_{53}NO_8$) in the portion of Moxidectin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Moxidectin RS in the *Standard solution* (mg/mL)

C_U = concentration of Moxidectin in the *Sample solution* (mg/mL)

Acceptance criteria: 92.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1, Jan-2018)

- **ORGANIC IMPURITIES: EARLY-ELUTING IMPURITIES**

Buffer, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

System suitability solution: 1.0 mg/mL of USP Moxidectin System Suitability Mixture RS in acetonitrile. Sonicate if necessary to facilitate dissolution.

Standard solution: 0.01 mg/mL of Moxidectin in acetonitrile from the *Sample solution*

System suitability

Sample: *System suitability solution*

Suitability requirements

Peak-to-valley ratio: NLT 3.0 between moxidectin 17a-epimer and moxidectin

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each early-eluting impurity in the portion of Moxidectin taken:

$$\text{Result} = (r_u/r_s) \times F \times D \times 100$$

r_u = peak response of each early-eluting impurity from the *Sample solution*

r_s = peak response of moxidectin from the *Standard solution*

F = Assay value expressed as a decimal

D = dilution factor used to prepare the *Standard solution*, 0.01

Acceptance criteria: See Table 1.

The reporting level for impurities is 0.1%. Disregard the peak due to the stabilizer (identify this peak, where applicable, by injecting a suitable reference solution).

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Moxidectin butenyl analog ^a	0.5	1.5
5'-Demethyl moxidectin ^b	0.7	0.5
Moxidectin pentenyl analog ^c	0.75	1.5
Moxidectin 17a-epimer ^d	0.9	2.5
Moxidectin	1.0	—
Sum of moxidectin 19-S-17a-ene ^e and moxidectin ethyl isomers ^f	1.3–1.5	1.7 ^h
Milbemycin B analog (moxidectin open ring) ^g	1.6	1.5
Any other individual impurity eluting before milbemycin B analog (moxidectin open ring)	—	0.5

^a (2aE,4E,5'R,6R,6'S,8E,11R,13S,15S,17aR,20R,20aR,20bS)-6'-[(E)-But-2-en-2-yl]-5',6,6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5',6,8,19-tetramethylspiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-4',17(3'H)-dione 4'-(E)-(O-methyloxime).

^b (2aE,4E,5'R,6R,6'S,8E,11R,13S,15S,17aR,20R,20aR,20bS)-5',6,6',7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20,20b-dihydroxy-6'-[(E)-4-methylpent-2-en-2-yl]-6,8,19-trimethylspiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-4',17(3'H)-dione 4'-(E)-(O-methyloxime).

^c (2aE,4E,5'R,6R,6'S,8E,11R,13S,15S,17aR,20R,20aR,20bS)-5',6,6',7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20,20b-dihydroxy-5',6,8,19-tetramethylpent-2-en-2-yl]-spiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-4',17(3'H)-dione 4'-(E)-(O-methyloxime).

^d (2aE,4E,5'R,6R,6'S,8E,11R,13S,15S,17aS,20R,20aR,20bS)-5',6,6',7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20,20b-dihydroxy-6'-[(E)-4-methylpent-2-en-2-yl]-5',6,8,19-tetramethylspiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-4',17(3'H)-dione 4'-(E)-(O-methyloxime).

^e (2aE,4E,5'R,6R,6'S,8E,11R,13S,15S,19S,20R,20aR,20bS)-5',6,6',7,10,11,14,15,19,20,20a,20b-Dodecahydro-20,20b-dihydroxy-6'-[(E)-4-methylpent-2-en-2-yl]-5',6,8,19-tetramethylspiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-4',17(3'H)-dione 4'-(E)-(O-methyloxime).

^f Mixture of five possible isomers, where one methyl group in the analyte is replaced with an ethyl group.

^g (2'R,3S,5'S,6'S,7R,9E,12R,13E,15E,16aS,18S,20aR)-16a,18-Dihydroxy-5',10,12,16,19-pentamethyl-6'-[(E)-4-methylpent-2-en-2-yl]-3,4,5',6',7,8,11,12,16a,17,18,20a-dodecahydro-1H-spiro[3,7-methanobenzo[g][1,5]dioxacyclooctadecin-5,2'-[2H]pyran]-14'-dione (E)-(O-methyloxime).

^h If present, moxidectin 19-S-17a-ene and the moxidectin ethyl isomers may not be completely resolved by the method. These peaks are integrated together to determine conformance.

- **ORGANIC IMPURITIES: LATE-ELUTING IMPURITIES**

Buffer: Dissolve 3.8 g of ammonium acetate in 250 mL of water, and adjust with glacial acetic acid to a pH of 4.2.

Mobile phase: Acetonitrile and *Buffer* (75:25)

System suitability solution: 3.0 mg/mL of USP Moxidectin System Suitability Mixture RS in acetonitrile.

Sonicate if necessary to facilitate dissolution.

Sample solution: 3.0 mg/mL of Moxidectin in acetonitrile. Sonicate if necessary to facilitate dissolution.

Standard solution: 0.03 mg/mL of Moxidectin in acetonitrile from the *Sample solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 3.9-mm × 15-cm; 4-μm packing L1

Column temperature: 35°

Flow rate: 2 mL/min

Injection volume: 10 μL

Run time: NLT 10 times the retention time of moxidectin

System suitabilitySample: *System suitability solution***Suitability requirements**

Resolution: NLT 1.0 between moxidectin deoxydiene/methylthiomethoxymoxidectin and 20b-methylthiomoxidectin

AnalysisSamples: *Standard solution* and *Sample solution*

Calculate the percentage of each late-eluting impurity in the portion of Moxidectin taken:

$$\text{Result} = (r_u/r_s) \times F \times D \times 100$$

 r_u = peak response of each late-eluting impurity from the *Sample solution* r_s = peak response of moxidectin from the *Standard solution* F = Assay value expressed as a decimal D = dilution factor used to prepare the *Standard solution*, 0.01

Acceptance criteria: See Table 2.

The reporting level for impurities is 0.1%. Disregard the peak due to the stabilizer (identify this peak, where applicable, by injecting a suitable reference solution).

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Moxidectin	1.0	—
Moxidectin deoxydiene ^a and 4'-Methylthiomethoxymoxidectin ^b	2.0	1.0 ^c
20b-Methylthiomoxidectin ^c	2.2	0.5
20-Nitrobenzoylmoxidectin ^d	3.4	0.5
Any other individual impurity eluting after the milbemycin B analog (moxidectin open ring) (≈ 1.4 RRT)	—	0.5

^a (2aE,4E,5'R,6R,6'S,8E,11R,13S,15S,20aR,20bS)-5',6,6',7,10,11,14,15,20a,20b-Decahydro-20b-hydroxy-6'-[(E)-4-methylpent-2-en-2-yl]-5',6,8,19-tetramethylspiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-4',17(3'H)-dione 4'-E-(O-methylxime).

^b (2aE,4E,5'S,5'R,6R,6'S,8E,11R,13S,15S,17aR,20R,20aR,20bS)-3',4',5',6,6',7,10,11,14,15,17a,20,20a,20b-Tetradecahydro-20,20b-dihydroxy-6'-[(E)-4-methylpent-2-en-2-yl]-4'-methylthiomethoxy-5',6,8,19-tetramethylspiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-17-one.

^c (2aE,4E,5'R,6R,6'S,8E,11R,13S,15S,17aR,20R,20aR,20bS)-5',6,6',7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20-hydroxy-6'-[(E)-4-methylpent-2-en-2-yl]-20b-methylthiomethoxy-5',6,8,19-tetramethylspiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-4',17(3'H)-dione 4'-E-(O-methylxime).

^d (2aE,4E,5'R,6R,6'S,8E,11R,13S,15S,17aR,20R,20aR,20bS)-5',6,6',7,10,11,14,15,17a,20,20a,20b-Dodecahydro-20b-hydroxy-6'-[(E)-4-methylpent-2-en-2-yl]-20-(4-nitrobenzoyloxy)-5',6,8,19-tetramethylspiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-4',17(3'H)-dione 4'-E-(O-methylxime).

^e If present, impurities moxidectin deoxydiene and 4'-methylthiomethoxymoxidectin may not be completely resolved by the method. These peaks are integrated together to determine conformance.

TOTAL ORGANIC IMPURITIESAnalysis: Calculate the sum of all impurities found in the tests for *Organic Impurities: Early-Eluting Impurities* and *Organic Impurities: Late-Eluting Impurities* in the portion of Moxidectin taken.

Acceptance criteria: NMT 7.0%

SPECIFIC TESTS• **WATER DETERMINATION, Method I (921):** NMT 1.3%**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store in a refrigerator.
- **LABELING:** Label it to indicate that it is for veterinary use only. Label it to state the name(s) and amount(s) of any added substance(s).

USP REFERENCE STANDARDS (11)

USP Moxidectin RS

USP Moxidectin System Suitability Mixture RS

Moxifloxacin Ophthalmic Solution**DEFINITION**Moxifloxacin Ophthalmic Solution is a sterile, self-preserved aqueous solution of Moxifloxacin Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of moxifloxacin ($C_{21}H_{24}FN_3O_4$).**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY**PROCEDURE**

Solution A: Dissolve 0.5 g of tetrabutylammonium hydrogen sulfate and 1.0 g of monobasic potassium phosphate in 1000 mL of water. Add 2 mL of phosphoric acid, filter, and degas.

Solution B: Methanol

Mobile phase: See Table 1.

Table 1

Time (min)	Flow Rate (mL/min)	Solution A (%)	Solution B (%)
0	0.5	69	31
30	0.5	69	31
31	0.9	60	40
36	0.9	60	40
37	0.5	69	31
42	0.5	69	31

System suitability solution: 0.1 mg/mL of USP Moxifloxacin Hydrochloride RS and 1 μ g/mL of USP Moxifloxacin Related Compound A RS in *Solution A*

Standard stock solution: 6 mg/mL of USP Moxifloxacin Hydrochloride RS in water

Standard solution: 0.1 mg/mL of USP Moxifloxacin Hydrochloride RS in *Solution A* from *Standard stock solution*Sample solution: Nominally 0.1 mg/mL of moxifloxacin from Ophthalmic Solution in *Solution A***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 293 nm

Column: 4.0-mm \times 25-cm; 5- μ m packing L11

Column temperature: 45°

Flow rate: See Table 1.

Injection volume: 25 μ L**System suitability**Samples: *System suitability solution* and *Standard solution***Suitability requirements**Resolution: NLT 2.0 between the moxifloxacin and moxifloxacin related compound A peaks, *System suitability solution*Column efficiency: NLT 4000 theoretical plates, *Standard solution*Tailing factor: NMT 2.0, *Standard solution*Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of moxifloxacin ($C_{21}H_{24}FN_3O_4$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response of moxifloxacin from the *Sample solution*
 r_S = peak response of moxifloxacin from the *Standard solution*
 C_S = concentration of USP Moxifloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of moxifloxacin in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of moxifloxacin, 401.43
 M_{r2} = molecular weight of moxifloxacin hydrochloride, 437.89

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **ORGANIC IMPURITIES: EARLY-ELUTING RELATED COMPOUNDS (RELATIVE RETENTION TIME LESS THAN 1.8)**

Protect the *System suitability solution*, *Standard solution*, and *Sample solution* from light. Analyze the *Sample solution* immediately after preparation.

Solution A: Dissolve 0.5 g of tetrabutylammonium hydrogen sulfate and 1.0 g of monobasic potassium phosphate in 1000 mL of water. Add 2 mL of phosphoric acid, filter, and degas.

Solution B: Methanol

Mobile phase: See Table 1.

Blank solution: Use *Solution A*.

System suitability solution: 0.1 mg/mL of USP Moxifloxacin Hydrochloride RS and 1 µg/mL of USP Moxifloxacin Related Compound A RS in *Solution A*

Standard stock solution: 6 mg/mL of USP Moxifloxacin Hydrochloride RS in water

Standard solution: 2 µg/mL of USP Moxifloxacin Hydrochloride RS in *Solution A* from *Standard stock solution*

Sensitivity solution: 0.05 µg/mL of USP Moxifloxacin Hydrochloride RS from the *Standard solution* in *Solution A*. Store the *Sensitivity solution* under refrigeration and protected from light.

Sample solution: Nominally 0.1 mg/mL of moxifloxacin from Ophthalmic Solution in *Solution A*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 293 nm

Column: 4.0-mm × 25-cm; 5-µm packing L11

Column temperature: 45°

Flow rate: See Table 1.

Injection volume: 25 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0 between the moxifloxacin and moxifloxacin related compound A peaks, *System suitability solution*

Column efficiency: NLT 4000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Blank solution*, *Standard solution*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

- r_U = peak response of each impurity from the *Sample solution*
 r_S = peak response of moxifloxacin from the *Standard solution*
 C_S = concentration of USP Moxifloxacin Hydrochloride RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of moxifloxacin in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of moxifloxacin, 401.43
 M_{r2} = molecular weight of moxifloxacin hydrochloride, 437.89
 F = relative response factor (see Table 2)
 Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Specified unknown impurity #1	0.3	1.0	0.2
Decarboxy ^a	0.4	0.13	0.3
Specified unknown impurity #2	0.9	1.0	0.3
Moxifloxacin	1.0	—	—
Moxifloxacin related compound A ^b	1.1	—	—
8-Hydroxy ^{c,d}	1.8	—	—
Other single impurities ^e	—	1.0	0.1
Any specified and identified impurity	—	1.0	1.0

^a 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4aS,7aS)-octahydro-pyrrolo[3,4-b]pyridin-6-yl]-1H-quinolin-4-one.

^b Disregard this peak because this is a process impurity controlled for the drug substance.

^c 1-Cyclopropyl-6-fluoro-8-hydroxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

^d Disregard this peak because it is quantitated using *Organic Impurities: Late-Eluting Related Compounds (Relative Retention Time Equal to More Than 1.8)*.

^e Disregard any peaks obtained from the *Blank*.

• **ORGANIC IMPURITIES: LATE-ELUTING RELATED COMPOUNDS (RELATIVE RETENTION TIME EQUAL TO MORE THAN 1.8)**

Protect the *Standard solution* and *Sample solution* from light. Analyze the *Sample solution* immediately after preparation.

Solution A: Dissolve 0.5 g of tetrabutylammonium hydrogen sulfate and 1.0 g of monobasic potassium phosphate in 1000 mL of water. Add 2 mL of phosphoric acid, filter, and degas.

Solution B: Methanol

Mobile phase: *Solution B* and *Solution A* (40:60)

Blank solution: Use *Solution A*.

Standard stock solution: 6 mg/mL of USP Moxifloxacin Hydrochloride RS in water

Standard solution: 2 µg/mL of USP Moxifloxacin Hydrochloride RS in *Solution A* from *Standard stock solution*

Sensitivity solution: 0.05 µg/mL of USP Moxifloxacin Hydrochloride RS from the *Standard solution* in *Solution A*. Store the *Sensitivity solution* under refrigeration and protected from light.

Sample solution: Nominally 0.1 mg/mL of moxifloxacin from Ophthalmic Solution in *Solution A*. The 8-hydroxy compound is unstable in dilute aqueous solutions. Analyze the *Sample solution* immediately after preparation.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 293 nm

Column: 4.0-mm × 25-cm; 5-μm packing L11

Column temperature: 45°

Flow rate: 0.9 mL/min

Injection volume: 25 μL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Blank solution*, *Standard solution*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of moxifloxacin from the *Standard solution*

C_S = concentration of USP Moxifloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of moxifloxacin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of moxifloxacin, 401.43

M_{r2} = molecular weight of moxifloxacin hydrochloride, 437.89

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Moxifloxacin	1.0	—	—
8-Hydroxy	1.8	0.29	0.2
Specified unknown impurity #3	3.4	1.0	0.2
Specified impurity #4 ^a	3.9	0.42	0.2
Other single impurities	—	1.0	0.1

^a 7-Amino-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.

Total impurities (sum from both *Organic Impurities* tests) is NMT 1.5%.

SPECIFIC TESTS

• **STERILITY TESTS** <71>: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.

• **PH** <791>: 6.3–7.3

• **OSMOLALITY AND OSMOLARITY** <785>: 260–320 mOsmol/kg

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 2° and 25°.

• **USP REFERENCE STANDARDS** (11)

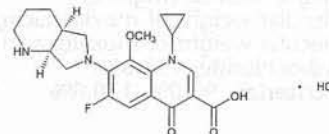
USP Moxifloxacin Hydrochloride RS

USP Moxifloxacin Related Compound A RS

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.

$C_{20}H_{21}F_2N_3O_3$ 389.40

Moxifloxacin Hydrochloride



$C_{21}H_{24}FN_3O_4 \cdot HCl$ 437.89

(4a*S*-*cis*)-1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl)-4-oxo-3-quinolinecarboxylic acid, monohydrochloride;

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid, monohydrochloride [186826-86-8].

DEFINITION

Moxifloxacin Hydrochloride contains NLT 98.0% and NMT 102.0% of moxifloxacin hydrochloride ($C_{21}H_{24}FN_3O_4 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION** <197K>

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

• **C. IDENTIFICATION TESTS—GENERAL** <191>, *Chloride*

Sample solution: To a solution (1 in 160) add diluted nitric acid, and filter.

Acceptance criteria: Meets the requirements

ASSAY

Change to read:

PROCEDURE

Buffer: Dissolve 0.5 g of tetrabutylammonium hydrogen sulfate and 1.0 g of monobasic potassium phosphate in water, add 2 mL of phosphoric acid, dilute with water to 1000 mL, mix, and pass through a filter of 0.45-μm pore size.

Mobile phase: Methanol and *Buffer* (28:72)

Diluent: Add 20 mg of anhydrous sodium sulfite to 1000 mL of *Buffer*, mix gently, and pass through a filter of 0.45-μm pore size.

System suitability solution: 0.1 mg/mL of USP Moxifloxacin Hydrochloride RS and 1 μg/mL of USP Moxifloxacin Related Compound A RS, in *Diluent*

Standard solution: 0.1 mg/mL of USP Moxifloxacin Hydrochloride RS in *Diluent*

Sample solution: 0.1 mg/mL of Moxifloxacin Hydrochloride in *Diluent*

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 293 nm

Column: 4.0-mm × 25-cm; 5-μm packing L11

Column temperature: 45°

Flow rate: 0.9 mL/min

Injection volume: 25 μL

System suitability

Samples: System suitability solution and Standard solution

[NOTE—The relative retention times for moxifloxacin and moxifloxacin related compound A are about 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 1.5 between moxifloxacin and moxifloxacin related compound A, System suitability solution

▲ USP40

Tailing factor: NMT 2.0, Standard solution

Relative standard deviation: ▲NMT 0.73%, ▲USP40 Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of moxifloxacin hydrochloride (C₂₁H₂₄N₃O₄ · HCl) in the portion of Moxifloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the Sample solution r_S = peak response from the Standard solution C_S = concentration of USP Moxifloxacin Hydrochloride RS in the Standard solution (mg/mL) C_U = concentration of Moxifloxacin Hydrochloride in the Sample solution (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

- **CHLORIDE AND SULFATE (221), Sulfate**

Sample: 0.6 g

Acceptance criteria: 0.04%; the Sample shows no more sulfate than corresponds to 0.25 mL of 0.020 N sulfuric acid.

Change to read:

- **ORGANIC IMPURITIES**

▲Protect all solutions containing moxifloxacin from light.

▲USP40

Mobile phase, Diluent, System suitability solution, and Sample solution: Prepare as directed in the Assay.

Blank solution: Use the Diluent.

Standard solution: 2 μg/mL of USP Moxifloxacin Hydrochloride RS in Diluent

Sensitivity solution: 0.05 μg/mL of USP Moxifloxacin Hydrochloride RS from the Standard solution in Diluent. Store the Sensitivity solution under refrigeration and protected from light.

Chromatographic system: Proceed as directed in the Assay with a run time of two times the retention time of moxifloxacin.

System suitability

Samples: System suitability solution, Standard solution, and Sensitivity solution

Suitability requirements

Resolution: NLT 1.5 between moxifloxacin and moxifloxacin related compound A, System suitability solution

▲USP40

Tailing factor: NMT 2.0, Standard solution

Relative standard deviation: NMT 2.0%, ▲USP40 Standard solution

Signal-to-noise ratio: NLT 10, Sensitivity solution

Analysis

Samples: Sample solution, Blank solution, and Standard solution

Calculate the percentage of each impurity in the portion of Moxifloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak response of each impurity from the Sample solution r_S = peak response of moxifloxacin from the Standard solution C_S = concentration of USP Moxifloxacin Hydrochloride RS in the Standard solution (mg/mL) C_U = concentration of Moxifloxacin Hydrochloride in the Sample solution (mg/mL) F = relative response factor (see Table 1)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Moxifloxacin	1.0	—	—
Moxifloxacin related compound A ^a	1.15	1.0	0.1
▲Moxifloxacin related compound B ^b ▲USP40 ^b	1.32	0.71	0.1
▲Moxifloxacin related compound C ^c ▲USP40 ^c	1.48	1.0	0.1
▲Moxifloxacin related compound D ^d ▲USP40 ^d	1.71	1.0	0.1
▲Moxifloxacin related compound E ^e ▲USP40 ^e	1.83	0.29	0.1
Other individual impurity	—	1.0	0.1
Total impurities	—	—	0.5

^a 1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.^b 1-Cyclopropyl-6,8-dimethoxy-1,4-dihydro-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.^c 1-Cyclopropyl-8-ethoxy-6-fluoro-1,4-dihydro-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.^d 1-Cyclopropyl-8-fluoro-6-methoxy-1,4-dihydro-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.^e 1-Cyclopropyl-6-fluoro-8-hydroxy-1,4-dihydro-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.**Add the following:**

- ▲**ENANTIOMERIC PURITY**

Protect all solutions containing moxifloxacin from light.

Buffer: 0.47 g/L of anhydrous cupric sulfate and 1.31 g/L of L-isoleucine in water. Adjust with 0.1 N sodium hydroxide to a pH of 4.50.

Solution A: Methanol and Buffer (500:1500)

Solution B: Methanol and Buffer (225:450)

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
50	100	0
51	0	100

Table 2 (Continued)

Time (min)	Solution A (%)	Solution B (%)
61	0	100
62	100	0
85	100	0

System suitability solution: 8 mg/mL of USP Moxifloxacin Hydrochloride RS and 8 µg/mL of USP Moxifloxacin Related Compound G RS in *Solution A*

Sensitivity solution: 0.8 µg/mL of USP Moxifloxacin Related Compound G RS in *Solution A*

Sample solution: 8 mg/mL of Moxifloxacin Hydrochloride in *Solution A*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 295 nm

Column: 3.0-mm × 15-cm; 3-µm packing L1

Flow rate: 0.42 mL/min

Injection volume: 1.5 µL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—The relative retention times for moxifloxacin related compound G and moxifloxacin are 0.78 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between moxifloxacin related compound G and moxifloxacin, *System suitability solution*

Signal-to-noise ratio: NLT 5, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of moxifloxacin related compound G in the portion of Moxifloxacin Hydrochloride taken:

$$\text{Result} = [r_u / (r_s + r_u)] \times 100$$

r_u = peak response of moxifloxacin related compound G from the *Sample solution*

r_s = peak response of moxifloxacin from the *Sample solution*

Acceptance criteria: NMT 0.10%▲USP40

SPECIFIC TESTS

Delete the following:

▲ OPTICAL ROTATION, *Specific Rotation* (781S)

Sample: 10 mg/mL in acetonitrile and water (1:1)

Acceptance criteria: −125° to −138° at 20°▲USP40

• MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62)

Total aerobic microbial count: NMT 10³ cfu/g

Total combined molds and yeasts count: NMT 10² cfu/g

• pH (791)

Sample solution: 2 mg/mL

Acceptance criteria: 3.9–4.6

• WATER DETERMINATION (921), *Method I*, *Method Ia*: NMT 4.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.

Change to read:

• USP REFERENCE STANDARDS (11)

USP Moxifloxacin Hydrochloride RS

USP Moxifloxacin Related Compound A RS

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid.

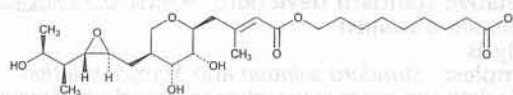
C₂₀H₂₁F₂N₃O₃ 389.40

▲USP Moxifloxacin Related Compound G RS

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4a*R*,7a*R*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid, monohydrochloride.

C₂₁H₂₄FN₃O₄ · HCl 437.89▲USP40

Mupirocin



C₂₆H₄₄O₉ 500.62

Nonanoic acid, 9-[[[3-methyl-1-oxo-4-[[tetrahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-1-methylpropyl)oxiran-yl]methyl]-2*H*-pyran-2-yl]-2-butenyl]oxy]-, [2*S*-2α(*E*), 3β,4β, 5α[2*R**, 3*R**(1*R**, [2*R**)]]]-

(*E*)-(2*S*,3*R*,4*R*,5*S*)-5-[[[2*S*,3*S*,4*S*,5*S*)-2,3-Epoxy-5-hydroxy-4-methylhexyl]tetrahydro-3,4-dihydroxy-β-methyl-2*H*-pyran-2-crotonic acid, ester with 9-hydroxynonanoic acid [12650-69-0].

» Mupirocin contains not less than 920 µg and not more than 1020 µg of mupirocin (C₂₆H₄₄O₉) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Mupirocin RS

USP Mupirocin Lithium RS

Identification—The IR absorption spectrum of a mineral oil dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Mupirocin RS.

Crystallinity (695): meets the requirements.

pH (791): between 3.5 and 4.5, in a saturated aqueous solution.

Water Determination, Method I (921): not more than 1.0%.

Assay—

pH 6.3 phosphate buffer—Prepare 0.05 M monobasic sodium phosphate, and adjust with 10 N sodium hydroxide to a pH of 6.3 ± 0.2.

Mobile phase—Prepare a suitable mixture of pH 6.3 phosphate buffer and acetonitrile (750:250), pass through a suitable filter of 0.5 µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Transfer about 11 mg of USP Mupirocin Lithium RS, accurately weighed, to a 100-mL volumetric flask, add 25 mL of acetonitrile, and swirl to dissolve. Dilute with pH 6.3 phosphate buffer to volume, and mix.

Resolution solution—Adjust 10 mL of *Standard preparation* with 6 N hydrochloric acid to a pH of 2.0, allow to stand for 2 hours, and adjust with 5 N sodium hydroxide to a pH of 6.3 ± 0.2.

Assay preparation—Transfer about 11 mg of Mupirocin, accurately weighed, to a 100-mL volumetric flask, add

25 mL of acetonitrile, and swirl to dissolve. Dilute with pH 6.3 phosphate buffer to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 229-nm detector and a 4.6-mm × 25-cm column that contains packing L1 based on spherical silica particles. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for the mupirocin acid hydrolysis product and 1.0 for mupirocin, and the resolution, R , between the mupirocin acid hydrolysis product and mupirocin is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2, the column efficiency is not less than 1500 theoretical plates when calculated by the formula:

$$5.545(t_r / W_{h/2})^2$$

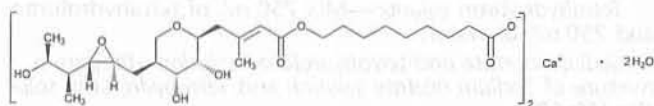
in which the terms are as defined therein. The relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in μ g, of mupirocin ($C_{26}H_{44}O_9$) in each mg of Mupirocin taken by the formula:

$$(M_s E / M_u)(r_u / r_s)$$

in which M_s is the weight, in mg, of USP Mupirocin Lithium RS taken to prepare the *Standard preparation*; E is the mupirocin equivalent, in μ g per mg, of USP Mupirocin Lithium RS; M_u is the weight, in mg, of mupirocin taken to prepare the *Assay preparation*; and r_u and r_s are the mupirocin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Mupirocin Calcium



$C_{52}H_{86}CaO_{18} \cdot 2H_2O$ 1075.34

Nonanoic acid, 9-[[[3-methyl-1-oxo-4-[tetrahydro-3,4-dihydroxy-5-[[[3-(2-hydroxy-1-methylpropyl)oxiranyl]methyl]-2H-pyran-2-yl]-2-butenyl]oxy-, calcium salt (2:1), dihydrate, [2S-[2 α (E), 3 β , 4 β , 5 α [2R*, 3R*(1R*, 2R*)]]]-; (α E, 2S, 3R, 4R, 5S)-5-[(2S, 3S, 4S, 5S)-2,3-Epoxy-5-hydroxy-4-methylhexyl]tetrahydro-3,4-dihydroxy- β -methyl-2H-pyran-2-crotonic acid, ester with 9-hydroxynonanoic acid, calcium salt (2:1), dihydrate [115074-43-6].

DEFINITION

Mupirocin Calcium contains the equivalent of NLT 865 μ g/mg and NMT 936 μ g/mg of mupirocin ($C_{26}H_{44}O_9$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
Sample: Do not grind extensively.
Acceptance criteria: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** (191), *Calcium*: Meets the requirements

ASSAY

• PROCEDURE

Solution A: 7.7 g/L of ammonium acetate in water, adjusted with glacial acetic acid to a pH of 5.7 before diluting to the final volume

Mobile phase: Tetrahydrofuran and *Solution A* (32:68)

Standard solution: 125 μ g/mL of USP Mupirocin Lithium RS prepared as follows. Transfer a suitable amount of USP Mupirocin Lithium RS to a suitable volumetric flask, dissolve in methanol, using 2.5% of the final volume, and dilute with *Solution A* to volume.

System suitability solution: Adjust 10 mL of the *Standard solution* with 6 N hydrochloric acid to a pH of 2.0, and allow to stand for 20 h.

Sample solution: Transfer 25 mg of Mupirocin Calcium to a 200-mL volumetric flask, dissolve in 5 mL of methanol, and dilute with *Solution A* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5- μ m packing L7

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 7.0 between the second of the two peaks corresponding to mupirocin rearrangement products and the peak corresponding to mupirocin, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in μ g/mg, of mupirocin ($C_{26}H_{44}O_9$) in the portion of Mupirocin Calcium taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times P$$

r_u = peak area of mupirocin from the *Sample solution*

r_s = peak area of mupirocin from the *Standard solution*

C_s = concentration of USP Mupirocin Lithium RS in the *Standard solution* (mg/mL)

C_u = concentration of Mupirocin Calcium in the *Sample solution* (mg/mL)

P = potency of mupirocin in USP Mupirocin Lithium RS (μ g/mg)

Acceptance criteria: 865–936 μ g/mg

IMPURITIES

• CHLORIDE AND SULFATE (221), *Chloride*

Analysis: Dissolve 50 mg in a mixture of 1 mL of 2 N nitric acid and 15 mL of methanol. Add 1 mL of silver nitrate TS.

Acceptance criteria: The turbidity does not exceed that produced by 0.70 mL of 0.020 N hydrochloric acid (0.5%).

• ORGANIC IMPURITIES

Solution A: Prepare as directed in the *Assay*.

Solution B: 13.6 g/L of sodium acetate in water, adjusted with glacial acetic acid to a pH of 4.0 before diluting to the final volume

Mobile phase: Tetrahydrofuran and *Solution A* (30:70)

Diluent: Methanol and *Solution B* (1:1)

Standard solution: 125 μ g/mL of USP Mupirocin Lithium RS in *Diluent*

System suitability solution: Adjust 10 mL of the *Standard solution* with 6 N hydrochloric acid to a pH of 2.0, allow to stand for 20 h, and adjust with 5 N sodium hydroxide to a pH of 4.0.

Sample solution: 5 mg/mL of Mupirocin Calcium in Diluent

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for two mupirocin rearrangement products and mupirocin in the *System suitability solution* are 0.63, 0.67, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 7.0 between the mupirocin rearrangement product, with a relative retention time of about 0.67, and mupirocin, *System suitability solution*

Column efficiency: NLT 3000 theoretical plates for the mupirocin peak, *Standard solution*

Tailing factor: NMT 2 for the mupirocin peak, *Standard solution*

Relative standard deviation: NMT 5% for the mupirocin peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each related compound in the portion of Mupirocin Calcium taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

r_u = peak area of any impurity from the *Sample solution*

r_s = peak area of mupirocin from the *Standard solution*

C_s = concentration of USP Mupirocin Lithium RS in the *Standard solution* (mg/mL)

C_u = concentration of Mupirocin Calcium in the *Sample solution* (mg/mL)

P = potency of mupirocin in USP Mupirocin Lithium RS (μg/mg)

F = conversion factor, 0.001 mg/μg

Acceptance criteria: See Table 1. Disregard any peak with an area less than 0.05 times the area of the mupirocin peak in the *Standard solution*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pseudomonic acid D ^a	0.75	2.5
Mupirocin	1.0	—
Any other unspecified impurity	—	1
Total impurities	—	4.5

^a (E)-9-[(E)-4-[(2S,3R,4R,5S)-3,4-Dihydroxy-5-[(2S,3S)-3-[(2S,3S)-3-hydroxybutan-2-yl]oxiran-2-yl)methyl]tetrahydro-2H-pyran-2-yl]-3-methylbut-2-enoyloxy]non-4-enoic acid.

SPECIFIC TESTS

• **OPTICAL ROTATION** (781S), *Specific Rotation*

Sample solution: 50 mg/mL in methanol

Acceptance criteria: −16° to −20°

• **WATER DETERMINATION** (921), *Method I*: 3.0%–4.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

• **USP REFERENCE STANDARDS** (11)

USP Mupirocin Calcium RS

USP Mupirocin Lithium RS

Mupirocin Cream

» Mupirocin Cream contains a quantity of Mupirocin Calcium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of mupirocin (C₂₆H₄₄O₉). It may contain one or more suitable buffers, dispersants, and preservatives.

Packaging and storage—Preserve in collapsible tubes or well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

Labeling—Label it to indicate that it contains Mupirocin Calcium and its equivalent content of mupirocin.

USP Reference standards (11)—

USP Mupirocin Lithium RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Minimum fill (755): meets the requirements.

pH (791): between 6.0 and 8.0.

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The total aerobic microbial count does not exceed 100 cfu per g.

Related compounds—

0.1 M Ammonium acetate, *Solution A*, *Solution B*, *Mobile phase*, and pH 6.3 Phosphate buffer—Proceed as directed in the *Assay*.

Sodium acetate solution—Add 5.8 mL of glacial acetic acid to 900 mL of water, adjust with sodium hydroxide TS to a pH of 4.0, dilute with water to 1000 mL, and mix.

Tetrahydrofuran solution—Mix 750 mL of tetrahydrofuran and 250 mL of water.

Sodium acetate and tetrahydrofuran solution—Prepare a mixture of *Sodium acetate solution* and *Tetrahydrofuran solution* (50:50).

System suitability solution—Use the *Standard preparation*, prepared as directed in the *Assay*.

Test stock solution—Transfer an accurately weighed quantity of Cream, equivalent to about 50 mg of mupirocin, to a screw-capped centrifuge tube. Add 5.0 mL of *Tetrahydrofuran solution*, cap, and disperse the Cream by mixing on a vortex mixer and shaking. Add 5.0 mL of *Sodium acetate solution*, cap, and mix. Centrifuge for about 15 minutes. Withdraw the lower layer from the tube, pass it through a filter having a 0.5-μm or finer porosity, and use the filtrate.

Test solution—Transfer 1.0 mL of the *Test stock solution* to a 50-mL volumetric flask, dilute with *Sodium acetate and tetrahydrofuran solution* to volume, mix, and pass through a filter having a 0.5-μm or finer porosity.

Chromatographic system (see *Chromatography* (621))—Prepare as directed in the *Assay*. Chromatograph the *Test stock solution*, and record the responses as directed for *Procedure*. Identify the peaks based on the relative retention times for mupirocin and related substances shown in Table 1: the resolution, R , between pseudomonic acid D and mupirocin is not less than 3. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the column efficiency for the mupirocin peak

is not less than 7000 theoretical plates; the tailing factor for the mupirocin peak is not more than 1.75; and the relative standard deviation of the mupirocin peak for replicate injections is not more than 2%.

Procedure—[NOTE—Ensure that buffers, dispersants, or preservatives in the formulation do not interfere with quantification of either impurities or degradation products.] Separately inject equal volumes (about 20 µL) of the *Test stock solution* and the *Test solution* into the chromatograph, and measure the peak responses for all of the peaks that do not correspond to buffers, dispersants, or preservatives. Calculate the percentage of each related compound and degradation product relative to mupirocin in the portion of Cream taken by the formula:

$$(r_i / r_M)(100 / 50)$$

in which r_i is the peak response for each related compound or degradation product obtained from the *Test stock solution*; r_M is the peak response of the mupirocin peak obtained from the *Test solution*; and 50 is the dilution factor for the *Test solution*.

Table 1

Name	Relative Retention Time	Limit (%)
Pseudomonic acid F ¹	0.36	NMT 1.2
Mupirocin impurity 1 ²	0.6	NMT 8.5
Mupirocin impurity 2 ³	0.63	NMT 16
Pseudomonic acid D ⁴	0.75	NMT 3.0
Pseudomonic acid B ⁵	0.9	NMT 1.2
Mupirocin	1.0	—
Mupirocin impurity 3 ⁶	1.15	NMT 1.2
Mupirocin impurity 4 ⁶	1.23	NMT 1.2
Pseudomonic acid C ⁷	2.03	NMT 1.2
Pseudomonic acid E ⁸	2.24	NMT 1.2
Any other unspecified impurity	—	NMT 1.2
Total impurities	—	NMT 30

¹ 7-[(E)-4-[(2S,3R,4R,5S)-3,4-Dihydroxy-5-[(2S,3S)-3-[(2S,3S)-3-hydroxybutan-2-yl]oxiran-2-yl)methyl]tetrahydro-2H-pyran-2-yl]-3-methylbut-2-enoyloxy]heptanoic acid.

² 9-[(E)-4-[(2R,3aS,6S,7S, 8aRS)-2-[(1RS,2S,3S)-1,3-Dihydroxy-2-methylbutyl]-7-hydroxyhexahydro-2H-furo[3,2-c]pyran-6-yl]-3-methylbut-2-enoyloxy]nonanoic acid.

³ 9-[(E)-4-[(2R,3RS,4aS,7S,8S,8aR)-3,8-Dihydroxy-2-[(2S,3S)-3-hydroxybutan-2-yl]octahydropyrano[3,2-c]pyran-7-yl]-3-methylbut-2-enoyloxy]nonanoic acid.

⁴ (E)-9-[(E)-4-[(2S,3R,4R,5S)-3,4-Dihydroxy-5-[(2S,3S)-3-[(2S,3S)-3-hydroxybutan-2-yl]oxiran-2-yl)methyl]tetrahydro-2H-pyran-2-yl]-3-methylbut-2-enoyloxy]non-4-enoic acid.

⁵ 9-[(E)-3-Methyl-4-[(2S,3R,4S,5R)-3,4,5-trihydroxy-5-[(2S,3S)-3-[(2S,3S)-3-hydroxybutan-2-yl]oxiran-2-yl)methyl]tetrahydro-2H-pyran-2-yl]but-2-enoyloxy]nonanoic acid.

⁶ 9-[(E)-4-[(2S,3R,4R,5S)-3,4-Dihydroxy-5-[(3-hydroxy-4,5-dimethyltetrahydrofuran-2-yl)methyl]tetrahydro-2H-pyran-2-yl]-3-methylbut-2-enoyloxy]nonanoic acid.

⁷ 9-[(E)-4-[(2S,3R,4R,5S)-3,4-Dihydroxy-5-[(4R,5S,E)-5-hydroxy-4-methylhex-2-enyl]tetrahydro-2H-pyran-2-yl]-3-methylbut-2-enoyloxy]nonanoic acid.

⁸ 11-[(E)-4-[(2S,3R,4R,5S)-3,4-Dihydroxy-5-[(2S,3S)-3-[(2S,3S)-3-hydroxybutan-2-yl]oxiran-2-yl)methyl]tetrahydro-2H-pyran-2-yl]-3-methylbut-2-enoyloxy]undecanoic acid.

Assay—

0.1 M Ammonium acetate—Dissolve about 7.7 g of ammonium acetate in about 900 mL of water in a 1000-mL volumetric flask, adjust with glacial acetic acid to a pH of 5.7, and dilute with water to volume.

Solution A—Prepare a filtered and degassed mixture of 0.1 M Ammonium acetate and tetrahydrofuran (75:25).

Solution B—Prepare a filtered and degassed mixture of 0.1 M Ammonium acetate and tetrahydrofuran (70:30):

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

pH 6.3 Phosphate buffer—Dissolve 69 g of monobasic sodium phosphate in 800 mL of water, adjust with sodium hydroxide TS to a pH of 6.3, dilute with water to 1000 mL, and mix.

Standard preparation—Dissolve an accurately weighed portion of USP Mupirocin Lithium RS in pH 6.3 Phosphate buffer. Dilute an accurately measured volume of this solution quantitatively with the same solvent to obtain a solution having a known concentration of about 0.1 mg of mupirocin per mL.

Assay preparation—Transfer an accurately weighed quantity of Cream, equivalent to about 10 mg of mupirocin, to a 100-mL volumetric flask. Add 50 mL of pH 6.3 Phosphate buffer and 25 mL of tetrahydrofuran. Insert the stopper into the flask, mix on a vortex mixer, and shake for 1 to 3 minutes. Dilute with pH 6.3 Phosphate buffer to volume. Allow to stand until the oil layer separates out, then dilute the aqueous layer with pH 6.3 Phosphate buffer to volume. Repeat 2 to 3 times until as much of the oil layer has separated out as possible. After the final dilution, pass the final solution (bottom layer) through a filter having a 0.5-µm or finer porosity. This solution will have a nominal concentration of 0.1 mg per mL of mupirocin based on label claim.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7. The flow rate is about 1 mL per minute. Maintain the column at a constant temperature up to 35°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–6	100	0	isocratic
6–35	100→0	0→100	linear gradient
35–55	0	100	isocratic
55–55.01	0→100	100→0	immediate
55.01–65	100	0	isocratic

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*. [NOTE—Pseudomonic acid D is a minor component that is always present in mupirocin calcium.] Identify the peaks by their retention times which are about 0.75 for pseudomonic acid D and 1.0 for mupirocin: the resolution, R , between pseudomonic acid D and mupirocin is not less than 3; the column efficiency for the mupirocin peak is not less than 7000 theoretical plates; the tailing factor for the mupirocin peak is not more than 1.75; and the relative standard deviation of the mupirocin peak for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak area responses for the major peaks. Calculate the percent label claim of mupirocin in the portion of Cream taken by the formula:

$$(P / 1000)(C_S / C_U)(r_U / r_S)(100)$$

in which $P / 1000$ is the potency of mupirocin, converted from µg per mg to mg per mg, in USP Mupirocin Lithium RS; C_S is the concentration, in mg per mL, of USP Mupirocin Lithium RS in the *Standard preparation*; C_U is the nominal concentration, in mg per mL, of Cream in the *Assay preparation*; and r_U and r_S are the mupirocin peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Mupirocin Ointment

» Mupirocin Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mupirocin ($C_{26}H_{44}O_9$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.

USP Reference standards (11)—

USP Mupirocin Lithium RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for mupirocin, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Minimum fill (755): meets the requirements.

Assay—

pH 6.3 phosphate buffer, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system—Proceed as directed in the *Assay* under Mupirocin.

Assay preparation—Dissolve an accurately weighed quantity of Ointment, equivalent to about 10 mg of mupirocin, in 25 mL of acetonitrile. Transfer this solution, with the aid of *pH 6.3 phosphate buffer*, to a 100-mL volumetric flask, dilute with *pH 6.3 phosphate buffer* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under Mupirocin. Calculate the quantity, in mg, of mupirocin ($C_{26}H_{44}O_9$) in the portion of Ointment taken by the formula:

$$(M_s E / 1000)(r_u / r_s)$$

in which the terms are as defined therein.

Mupirocin Nasal Ointment

» Mupirocin Nasal Ointment contains a quantity of Mupirocin Calcium equivalent to not less than 90.0 percent and not more than 105.0 percent of the labeled amount of mupirocin ($C_{26}H_{44}O_9$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers, and store at controlled room temperature.

USP Reference standards (11)—

USP Mupirocin Lithium RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—The total aerobic microbial count does not exceed 100 cfu per g, and the total combined molds and yeasts count does not exceed 10 cfu per g. It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Minimum fill (755): meets the requirements.

Uniformity of dosage units (905): meets the requirements.

Related compounds—

Ammonium acetate buffer, Mobile phase, Sodium acetate buffer, Diluent A, Diluent B, and Chromatographic system—Prepare as directed in the *Assay*.

Test solution—Transfer an accurately weighed portion of Mupirocin Nasal Ointment, equivalent to about 50 mg of mupirocin, to a suitable stoppered conical flask, add 5 mL of *Diluent A*, and shake vigorously on a mechanical shaker at full speed for 1 hour to disperse the ointment. Add 5 mL of *Sodium acetate buffer*, and shake vigorously on a mechanical shaker at full speed for 15 minutes. Pass through a filter having a porosity of 0.45 μ m.

Diluted test solution—Dilute a portion of the *Test solution* quantitatively, and stepwise if necessary, with *Diluent B* to obtain a solution having a nominal concentration of about 0.1 mg of mupirocin per mL, based on the label claim.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Test solution* and the *Diluted test solution* into the chromatograph, and measure the peak area responses for all the peaks. Identify the peaks by the relative retention times shown in Table 1.

Table 1

Name	Relative Retention Time	Limit (%)
Pseudomonic acid F ¹	0.32	1.0
Mupirocin impurity 1 ²	0.56	5.0
Mupirocin impurity 2 ³	0.60	5.0
Pseudomonic acid D ⁴	0.72	4.0
Pseudomonic acid B ⁵	0.88	1.0
Mupirocin	1.0	—
Mupirocin impurity 3 ⁶	1.24	1.0
Mupirocin impurity 4 ⁶	1.36	1.0
Mupirocin impurity 5 ⁷	2.80	1.0
and pseudomonic acid C ⁸		
Any individual unspecified impurity	—	0.5
Total impurities	—	10.0

¹7-((E)-4-((2S,3R,4R,5S)-3,4-Dihydroxy-5-((2S,3S)-3-((2S,3S)-3-hydroxybutan-2-yl)oxiran-2-yl)methyl)tetrahydro-2H-pyran-2-yl)-3-methylbut-2-enoyloxy)heptanoic acid.

²9-((E)-4-((2R,3aS,6S,7S, 8aRS)-2-((1R,2S,3S)-1,3-Dihydroxy-2-methylbutyl)-7-hydroxyhexahydro-2H-furo[3,2-c]pyran-6-yl)-3-methylbut-2-enoyloxy)nonanoic acid.

³9-((E)-4-((2R,3RS,4aS,7S,8S,8aR)-3,8-Dihydroxy-2-((2S,3S)-3-hydroxybutan-2-yl)octahydro-pyrano[3,2-c]pyran-7-yl)-3-methylbut-2-enoyloxy)nonanoic acid.

⁴(E)-9-((E)-4-((2S,3R,4R,5S)-3,4-Dihydroxy-5-((2S,3S)-3-((2S,3S)-3-hydroxybutan-2-yl)oxiran-2-yl)methyl)tetrahydro-2H-pyran-2-yl)-3-methylbut-2-enoyloxy)non-4-enoic acid.

⁵9-((E)-4-((2S,3R,4R,5S)-3,4,5-trihydroxy-5-((2S,3S)-3-((2S,3S)-3-hydroxybutan-2-yl)oxiran-2-yl)methyl)tetrahydro-2H-pyran-2-yl)but-2-enoyloxy)nonanoic acid.

⁶9-((E)-4-((2S,3R,4R,5S)-3,4-Dihydroxy-5-((3-hydroxy-4,5-dimethyltetrahydrofuran-2-yl)methyl)tetrahydro-2H-pyran-2-yl)-3-methylbut-2-enoyloxy)nonanoic acid.

⁷9-((E)-4-((2S,3R,4R,5S)-5-((4S,5S)-2-Chloro-3,5-dihydroxy-4-methylhexyl)-3,4-dihydroxytetrahydro-2H-pyran-2-yl)-3-methylbut-2-enoyloxy)nonanoic acid.

⁸9-((E)-4-((2S,3R,4R,5S)-3,4-Dihydroxy-5-((4R,5S,E)-5-hydroxy-4-methylhex-2-enyl)tetrahydro-2H-pyran-2-yl)-3-methylbut-2-enoyloxy)nonanoic acid.

Calculate the percentage of each related compound in the portion of Mupirocin Nasal Ointment taken by the formula:

$$(100 \times r_i) / (D \times r_s)$$

in which r_i is the peak response of any impurity in the *Test solution*; D is the dilution factor used to convert the *Test solution* to the *Diluted test solution*; and r_s is the peak response for the mupirocin peak in the *Diluted test solution*. The specified and unspecified impurities meet the limits listed in Table 1.

Assay—

Ammonium acetate buffer—Dissolve about 7.7 g of ammonium acetate in water, and dilute with water to 1000 mL.

Mix, and adjust with acetic acid to a pH of 5.7. Filter this solution prior to preparation of the *Mobile phase*.

Mobile phase—Prepare a mixture of *Ammonium acetate buffer* and tetrahydrofuran (75 : 25). Make adjustments if necessary (see *System suitability* under *Chromatography* (621)). The *Mobile phase* is extremely sensitive to changes in tetrahydrofuran concentration. Degas the *Mobile phase* by helium sparging or ultrasonication before use.

Sodium acetate buffer—Dissolve about 13.6 g of sodium acetate in water, and dilute with water to 1000 mL. Mix, and adjust with acetic acid to a pH of 4.0.

Diluent A: a mixture of tetrahydrofuran and water (75:25).

Diluent B: a mixture of *Diluent A* and *Sodium acetate buffer* (1:1).

Standard preparation—Prepare a solution of USP Mupirocin Lithium RS in *Diluent B*, containing about 0.1 mg per mL of mupirocin.

Assay preparation—Transfer an accurately weighed portion of Mupirocin Nasal Ointment, equivalent to about 10 mg of mupirocin, to a 100-mL volumetric flask, add 50.0 mL of *Diluent A*, and shake vigorously on a mechanical shaker at full speed for 1 hour to disperse the ointment. Dilute with *Sodium acetate buffer* to volume, and shake vigorously on a mechanical shaker at full speed for 15 minutes. Prior to use, pass through a filter having a porosity of 0.45 μ m.

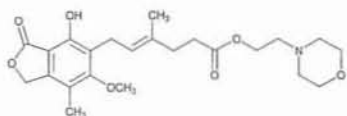
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm \times 25-cm column that contains 7- μ m packing L7. The flow rate is about 1.5 mL per minute. [NOTE—The flow rate may be adjusted if needed to obtain a retention time of about 13 minutes for the mupirocin peak.] Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the peaks for pseudomonic acid D and mupirocin is not less than 3.5; the column efficiency for the mupirocin peak is not less than 3000 theoretical plates; the tailing factor for the mupirocin peak is not more than 2.0; and the relative standard deviation of the mupirocin peak for five replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and measure the responses for the major peaks. Calculate the percentage of the label claim of mupirocin in the portion of Mupirocin Nasal Ointment taken by the formula:

$$(P / 1000)(C_s / C_u)(r_u / r_s)(100)$$

in which (*P* / 1000) is the potency, converted from μ g per mg to mg per mg, of mupirocin in USP Mupirocin Lithium RS; *C*_s is the concentration, in mg per mL, of mupirocin in the *Standard preparation*; *C*_u is the nominal concentration, in mg per mL, of mupirocin in the *Assay preparation*; and *r*_u and *r*_s are the peak responses for the mupirocin peak in the *Assay preparation* and the *Standard preparation*, respectively.

Mycophenolate Mofetil



C₂₃H₃₁NO₇

433.49

4-Hexenoic acid, 6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-, 2-(4-morpholinyl)ethyl ester, (E)-;
2-Morpholinoethyl (E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoate [128794-94-5].

DEFINITION

Mycophenolate Mofetil contains NLT 98.0% and NMT 102.0% of mycophenolate mofetil (C₂₃H₃₁NO₇), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Buffer: Triethylamine and water (1:325). Adjust with phosphoric acid to a pH of 5.3.

Mobile phase: Acetonitrile and *Buffer* (7:13)

Standard solution: 0.4 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile

Sample solution: 0.4 mg/mL of Mycophenolate Mofetil in acetonitrile

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Column temperature: 45°

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mycophenolate mofetil (C₂₃H₃₁NO₇) in the portion of Mycophenolate Mofetil taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times 100$$

*r*_u = peak response from the *Sample solution*

*r*_s = peak response from the *Standard solution*

*C*_s = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)

*C*_u = concentration of Mycophenolate Mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm (Official 1: Jan-2018)

ORGANIC IMPURITIES

Buffer and Mobile phase: Prepare as directed in the *Assay*.

Sample solution: 2 mg/mL of Mycophenolate Mofetil in acetonitrile

System suitability solution: 10 μ g/mL each of USP Mycophenolate Mofetil Related Compound A RS and USP Mycophenolate Mofetil Related Compound B RS in acetonitrile

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 250 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L7**Column temperature:** 45°**Flow rate:** 1.5 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *System suitability solution***Suitability requirements****Resolution:** NLT 1.5 between mycophenolate mofetil related compound A and mycophenolate mofetil related compound B**Analysis****Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Mycophenolate Mofetil taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 r_U = peak response for each impurity r_T = sum of all the peak responses**Acceptance criteria:** See *Table 1*. Disregard any peak less than 0.03%.**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mycophenolic acid ^a	0.33	0.50
Mycophenolate mofetil related compound A ^b	0.45	0.10
Mycophenolate mofetil related compound B ^c	0.49	0.10
N-Oxide analog ^d	0.60	0.10
1-Morpholinoethoxy analog ^e	0.86	0.10
Mycophenolate mofetil	1.0	—
Z-Mycophenolate mofetil ^f	1.1	0.10
O-Methyl analog ^g	1.2	0.10
Methyl mycophenolate ^h	1.5	0.10
Any single unspecified impurity	—	0.10
Total impurities	—	0.70

^a (E)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.^b 2-Morpholinoethyl (E)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.^c (RS)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3H-isobenzofuran-1-one.^d 2-Morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate N-oxide.^e 2-Morpholinoethyl (RS)-(E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-1-(2-morpholinoethoxy)-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.^f 2-Morpholinoethyl (Z)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.^g 2-Morpholinoethyl (E)-6-(1,3-dihydro-4,6-dimethoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.^h Methyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.**SPECIFIC TESTS****• LOSS ON DRYING (731)****Analysis:** Dry a sample under vacuum at 60° for 3 h.**Acceptance criteria:** NMT 0.5%**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.

• USP REFERENCE STANDARDS (11)

USP Mycophenolate Mofetil RS

USP Mycophenolate Mofetil Related Compound A RS
2-Morpholinoethyl (E)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.C₂₃H₃₁NO₇ 419.47USP Mycophenolate Mofetil Related Compound B RS
(RS)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3H-isobenzofuran-1-one.C₁₇H₂₀O₆ 320.34**Mycophenolate Mofetil Capsules****DEFINITION**Mycophenolate Mofetil Capsules contain NLT 94.0% and NMT 105.0% of the labeled amount of mycophenolate mofetil (C₂₃H₃₁NO₇).**IDENTIFICATION****• A. ULTRAVIOLET ABSORPTION (197U)****Standard solution and Sample solution:** Use the *Standard solution* and the *Sample solution* as prepared in *Dissolution Test 1*.**Acceptance criteria:** The UV absorption spectra of the *Standard solution* and the *Sample solution* exhibit maxima and minima at the same wavelength within ±3 nm.

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE****Phosphoric acid solution:** Phosphoric acid and water (3:50)**Triethylamine solution:** Transfer 3 mL of triethylamine to 1000 mL of water. Adjust with *Phosphoric acid solution* to a pH of 5.3.**Mobile phase:** Acetonitrile and *Triethylamine solution* (11:9)**Standard solution:** 0.125 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile**Sample solution:** Open Capsules, equivalent to 1.25 g of mycophenolate mofetil based on the label claim, and transfer the contents including Capsule shells into a 500-mL volumetric flask. Add 50 mL of water and shake mechanically for a minimum of 15 min. Add 350 mL of acetonitrile, sonicate for 15 min, and shake mechanically for 20 min. Dilute with acetonitrile to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask and dilute with acetonitrile to volume. Pass through a nylon filter of 0.45-μm pore size and discard the first 5 mL of the filtrate.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 250 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L7**Temperatures****Column:** 45°**Autosampler:** 10 ± 5°**Flow rate:** 1.5 mL/min**Injection volume:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of mycophenolate mofetil (C₂₃H₃₁NO₇) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL) C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)**Acceptance criteria:** 94.0%–105.0%**PERFORMANCE TESTS****• DISSOLUTION (711)****Test 1****Medium:** 0.1 N hydrochloric acid; 900 mL**Apparatus 2:** 40 rpm, with sinkers**Time:** 20 min**Standard solution:** 0.278 mg/mL of USP Mycophenolate Mofetil RS in *Medium***Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.**Detector:** UV 250 nm**Path length:** 0.1 cm**Blank:** *Medium***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of mycophenolate mofetil (C₂₃H₃₁NO₇) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of the *Standard solution* (mg/mL) V = volume of *Medium*, 900 mL L = label claim (mg/Capsule)**Tolerances:** NLT 80% (Q) of the labeled amount of mycophenolate mofetil (C₂₃H₃₁NO₇) is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.**Medium:** 0.1 N hydrochloric acid; 900 mL**Apparatus 2:** 40 rpm, with sinker**Time:** 30 min**Standard solution:** 0.028 mg/mL of USP Mycophenolate Mofetil RS in *Medium***Sample solution:** Pass a portion of the solution under test through a suitable nylon filter of 0.45-μm pore size. Discard the first 3–5 mL of the filtrate. Dilute 1 mL of the filtrate with *Medium* to 10 mL.**Instrumental conditions**(See *Ultraviolet-Visible Spectroscopy* (857).)**Mode:** UV**Analytical wavelength:** 250 nm**Blank:** *Medium***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of mycophenolate mofetil (C₂₃H₃₁NO₇) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of the *Standard solution* (mg/mL) V = volume of *Medium*, 900 mL D = dilution factor, 10 L = label claim (mg/Capsule)**Tolerances:** NLT 80% (Q) of the labeled amount of mycophenolate mofetil (C₂₃H₃₁NO₇) is dissolved.**• UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**IMPURITIES****• LIMIT OF DEGRADATION PRODUCTS****Mobile phase, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.**Sensitivity solution:** 0.0625 μg/mL of USP Mycophenolate Mofetil RS in acetonitrile**System suitability****Samples:** *Standard solution* and *Sensitivity solution***Suitability requirements****Tailing factor:** NMT 2, *Standard solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Signal-to-noise ratio:** NLT 10, *Sensitivity solution***Analysis**[NOTE—The run time for the *Sample solution* is three times that of the retention time of the mycophenolate mofetil peak.]**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak response of each individual impurity from the *Sample solution* r_S = peak response of mycophenolate mofetil from the *Standard solution* C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL) C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL) F = relative response factor for each individual impurity (see *Table 1*)**Acceptance criteria:** See *Table 1*. Disregard peaks at relative retention times of 1.45 and 2.15. Disregard any peaks less than 0.05%.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mycophenolic acid ^a	0.6	1.4	1.0
Mycophenolate N-oxide analog ^b	0.8	1.0	0.2

^a (E)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuran-4-yl)-4-methyl-4-hexenoic acid.^b 2-Morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuran-4-yl)-4-methyl-4-hexenoate N-oxide.

Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mycophenolate mofetil	1.0	—	—
Any single unspecified impurity	—	1.0	0.1
Total degradation products	—	—	1.5

^a (E)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.

^b 2-Morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate N-oxide.

• LIMIT OF Z-MYCOPHENOLATE MOFETIL

[NOTE—Z-Mycophenolate mofetil is 2-morpholinoethyl (Z)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoate.]

Triethylamine solution: Prepare as directed in the Assay.

Mobile phase: Acetonitrile and Triethylamine solution (7:13)

Sensitivity solution: 1.25 µg/mL of USP Mycophenolate Mofetil RS in acetonitrile

Standard solution: 0.025 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile

Sample solution: Open Capsules, equivalent to 1.25 g of mycophenolate mofetil based on the label claim, and transfer the contents including Capsule shells into a 500-mL volumetric flask. Add 50 mL of water and shake mechanically for a minimum of 15 min. Add 350 mL of acetonitrile, sonicate for 15 min, and shake mechanically for 20 min. Dilute with acetonitrile to volume. Pass through a nylon filter of 0.45-µm pore size and discard the first 2 mL of the filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Column temperature: 60°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: 1.7 times the retention time of the mycophenolate mofetil peak

System suitability

Samples: Sensitivity solution and Standard solution

[NOTE—The relative retention times for mycophenolate mofetil and Z-mycophenolate mofetil are 1.0 and 1.1, respectively.]

Suitability requirements

Tailing factor: NMT 2.0, Standard solution

Relative standard deviation: NMT 5.0%, Standard solution

Signal-to-noise ratio: NLT 10, Sensitivity solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of Z-mycophenolate mofetil in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of Z-mycophenolate mofetil from the Sample solution

r_S = peak response of mycophenolate mofetil from the Standard solution

C_S = concentration of USP Mycophenolate Mofetil RS in the Standard solution (mg/mL)

C_U = nominal concentration of mycophenolate mofetil in the Sample solution (mg/mL)

Acceptance criteria: NMT 0.10%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one Dissolution test is given, the labeling states the test used only if Test 1 is not used.
- **USP REFERENCE STANDARDS (11)**
USP Mycophenolate Mofetil RS

Mycophenolate Mofetil for Injection

DEFINITION

Mycophenolate Mofetil for Injection contains an amount of Mycophenolate Mofetil Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$).

IDENTIFICATION

- The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

ASSAY

• PROCEDURE

[NOTE—Protect solutions from light.]

Buffer 1: Transfer 10 mL of triethylamine into a 1000-mL volumetric flask containing about 950 mL of water and mix. Adjust with phosphoric acid to a pH of 7.2, and dilute with water to volume.

Buffer 2: Transfer 10 mL of triethylamine into a 1000-mL volumetric flask containing about 950 mL of water and mix. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to volume.

Solution A: Buffer 1 and water (4:9)

Diluent: Acetonitrile, Buffer 2, and water (7:4:9)

Mobile phase: Acetonitrile and Solution A (3:7)

Standard stock solution: Transfer a known quantity of USP Mycophenolate Mofetil RS in a suitable volumetric flask, add acetonitrile equivalent to about 10% of the final volume, and sonicate for about 5 min or until the solid dissolves. Dilute with Diluent to volume to obtain a solution containing a known concentration of 1.0 mg/mL of USP Mycophenolate Mofetil RS.

Standard solution: 0.4 mg/mL of USP Mycophenolate Mofetil RS in Diluent, from Standard stock solution

Sample solution: Constitute each of the containers of Mycophenolate Mofetil for Injection with 14 mL of 5% Dextrose Injection. Quantitatively transfer the contents of all vials, the combined contents of which are equivalent to about 2 g of mycophenolate mofetil, to a 200-mL volumetric flask, and dilute with water to volume. Transfer 4.0 mL of this solution to a 100-mL volumetric flask, and dilute with Diluent to volume.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 249 nm

Column: 4.6-mm × 25-cm; 5-μm packing L11

Temperature

Column: 45°

Autosampler: 5°

Flow rate: 1.5 mL/min

Injection size: 10 μL

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of $C_{23}H_{31}NO_7$ in the portion of Mycophenolate Mofetil for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of mycophenolate mofetil in the *Standard solution* (mg/mL) C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

IMPURITIES**Organic Impurities**

[NOTE—Protect solutions from light.]

• **PROCEDURE**Mobile phase, *Standard solution*, *Sample solution* and *Chromatographic system*: Proceed as directed in the *Assay*.System suitability solution: 0.01 mg/mL of USP Mycophenolate Mofetil Related Compound A RS and 0.01 mg/mL of USP Mycophenolate Mofetil Related Compound B RS in *Diluent*Sensitivity solution: 0.2 μg/mL in *Diluent*, from the *Standard solution***System suitability**Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

[NOTE—The relative retention times for mycophenolate mofetil related compound A and mycophenolate mofetil related compound B are 0.40 and 0.46, respectively, measured with respect to mycophenolate mofetil.]

Suitability requirementsResolution: NLT 2.0 between mycophenolate mofetil related compound A and mycophenolate mofetil related compound B, *System suitability solution*Signal-to-noise ratio: NLT 10, *Sensitivity solution*Tailing factor: NMT 2.0, *Standard solution*Relative standard deviation: NMT 2.0%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*[NOTE—The run time for the *Sample solution* is NLT 1.5 times the retention time of the mycophenolate mofetil peak.]

Calculate the percentage of each impurity in the portion of Mycophenolate Mofetil for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak response of each individual impurity from the *Sample solution* r_S = peak response of mycophenolate mofetil from the *Standard solution* C_S = concentration of mycophenolate mofetil in the *Standard solution* (mg/mL) C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL) F = relative response factor for each individual impurity (see *Impurity Table 1*)**Acceptance criteria**Individual impurities: See *Impurity Table 1*. [NOTE—Disregard any impurity peak less than 0.05%.]

Total impurities: NMT 1.35%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mycophenolic acid ^a	0.12	1.4	1.1
Mycophenolate mofetil	1.00	—	—
Any unspecified impurity	—	1.0	0.1

^a (E)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST** (85): Contains NMT 0.4 USP Endotoxin Unit/mg of mycophenolate mofetil
- **STERILITY TESTS** (71): Meets the requirements when tested as directed in *Test for Sterility of the Product to be Examined*, *Membrane Filtration*
- **WATER DETERMINATION, Method 1a** (921): NMT 1.0%
- **PH** (791): Between 2.7 and 4.1, in a reconstituted solution
- **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections
- **CONSTITUTED SOLUTION**: At the time of use, meets the requirements under *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
 - USP Endotoxin RS
 - USP Mycophenolate Mofetil RS
 - USP Mycophenolate Mofetil Related Compound A RS
 - 2-Morpholinoethyl (E)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.
 - $C_{23}H_{31}NO_7$ 419.47
 - USP Mycophenolate Mofetil Related Compound B RS
 - (RS)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3H-isobenzofuranyl-1-one.
 - $C_{17}H_{26}O_6$ 320.34

Mycophenolate Mofetil for Oral Suspension

DEFINITIONMycophenolate Mofetil for Oral Suspension is a dry mixture of mycophenolate mofetil and one or more suitable buffers, colors, diluents, and flavors. It contains NLT 90.0% and NMT 110.0% of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$).

IDENTIFICATION

- The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

[NOTE—Protect solutions from light.]

Buffer 1: Pipet 10 mL of triethylamine into a 1000-mL volumetric flask containing about 950 mL of water, and mix. Adjust with phosphoric acid to a pH of 7.2, and dilute with water to volume.

Buffer 2: Pipet 10 mL of triethylamine into a 1000-mL volumetric flask containing about 950 mL of water, and mix. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to volume.

Solution A: *Buffer 1* and water (4:9)

Extraction solvent: Acetonitrile, *Buffer 2*, and water (13:4:9)

Diluent: Acetonitrile, *Buffer 2*, and water (7:4:9)

Mobile phase: Acetonitrile and *Solution A* (3:7)

Standard stock solution: 4 mg/mL of USP Mycophenolate Mofetil RS in *Extraction solvent*. Sonicate to aid the dissolution.

Standard solution: 0.4 mg/mL of USP Mycophenolate Mofetil RS in *Diluent*, from the *Standard stock solution*

Sample stock solution: Constitute Mycophenolate Mofetil for Oral Suspension as directed on the label.

Prepare a composite sample by mixing NLT 4 bottles of the constituted Mycophenolate Mofetil for Oral Suspension. Transfer a volume of the composite sample so obtained, equivalent to 800 mg of mycophenolate mofetil, to a 200-mL volumetric flask, and dilute with *Extraction solvent* to volume.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pass through a filter of 45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 249 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L11

Column temperature: 45°

Autosampler temperature: 5°

Flow rate: 1.5 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{23}H_{31}NO_7$ in the portion of Mycophenolate Mofetil for Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of mycophenolate mofetil in the *Standard solution* (mg/mL)

C_u = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)

[NOTE—Prepare all solutions in low-actinic glassware.]

Medium: 0.1 N hydrochloric acid; 900 mL, deaerated

Apparatus 2: 40 rpm

Time: 20 min

Standard solution: 0.278 mg/mL of USP Mycophenolate Mofetil RS in *Medium*

Sample solution: Reconstitute Mycophenolate Mofetil for Oral Suspension according to the labeling instructions. Shake well. Use a separate 3-mL syringe for each vessel. Withdraw 2 mL of suspension. Remove air bubbles from the syringe. Adjust the volume to 1.2 mL and accurately weigh the filled syringe. Operate the apparatus, holding the syringe above the surface of the medium, at a location that is halfway between the paddle shaft and the vessel wall. Carefully introduce the sample to the vessel over a 5–10 s period. Weigh the empty syringe and determine the weight of the sample (g). At the time specified, withdraw an aliquot and immediately pass through a suitable filter of 10- μ m pore size, discarding the first few mL.

Spectrometric conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 304 nm

Cell: 0.2 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of mycophenolate mofetil dissolved:

$$\text{Result} = (A_u/A_s) \times (C_s/L) \times (V_1/V_2) \times 100$$

A_u = absorbance of the *Sample solution*

A_s = absorbance of the *Standard solution*

C_s = concentration of mycophenolate mofetil in the *Standard solution* (mg/mL)

L = suspension label claim of mycophenolate mofetil (mg/mL)

V_1 = volume of *Medium*, 900 (mL)

V_2 = volume of sample (mL), weight (g) of the sample divided by the density of the suspension (g/mL)

Tolerances: NLT 80% (Q) of the labeled amount of mycophenolate mofetil is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

- **DELIVERABLE VOLUME** (698): Meets the requirements

IMPURITIES**Organic Impurities**

[NOTE—Protect solutions from light.]

• **PROCEDURE**

Mobile phase, Standard solution, Sample solution and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 0.01 mg/mL of USP Mycophenolate Mofetil Related Compound A RS and 0.01 mg/mL of USP Mycophenolate Mofetil Related Compound B RS in *Diluent*. [NOTE—The relative retention times for mycophenolate mofetil related compound A and mycophenolate mofetil related compound B are 0.40 and 0.46, respectively, measured with respect to mycophenolate mofetil.]

Sensitivity solution: 0.2 μ g/mL in *Diluent*, from the *Standard solution*

System suitability

Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0 between mycophenolate mofetil related compound A and mycophenolate mofetil related compound B, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—The run time for the *Sample solution* is NLT 1.5 times the retention time of the mycophenolate mofetil peak.]

Calculate the percentage of each impurity in the portion of Mycophenolate Mofetil for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of mycophenolate mofetil from the *Standard solution*

C_S = concentration of mycophenolate mofetil in the *Standard solution* (mg/mL)

C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

[NOTE—Disregard any unspecified impurity peaks less than 0.05%.]

Total impurities: NMT 3.8%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mycophenolic acid ^a	0.12	1.4	3.3
Sorbitol ester of mycophenolic acid ^b	0.24	0.77	0.2
Mycophenolate mofetil	1.00	—	—
Any individual unspecified impurity	—	1.0	0.1

^a (E)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.

^b Sorbitol (E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate.

SPECIFIC TESTS

- PH (791):** Between 6.0 and 7.0, in the suspension constituted as directed in the labeling

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

- USP REFERENCE STANDARDS (11)**

USP Mycophenolate Mofetil RS

USP Mycophenolate Mofetil Related Compound A RS
2-Morpholinoethyl (E)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.

$C_{23}H_{31}NO_7$ 419.47

USP Mycophenolate Mofetil Related Compound B RS
(RS)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3H-isobenzofuranyl-1-one.

$C_{17}H_{20}O_6$ 320.34

Mycophenolate Mofetil Tablets

DEFINITION

Mycophenolate Mofetil Tablets contain NLT 94.0% and NMT 105.0% of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$).

IDENTIFICATION

- A. ULTRAVIOLET ABSORPTION (197U)**

Standard solution and Sample solution: Use the *Standard solution* and *Sample solution* as prepared in the test for *Dissolution*.

Acceptance criteria: The UV absorption spectrum of the *Standard solution* and the *Sample solution* exhibit maxima and minima at the same wavelength within ± 3 nm.

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- PROCEDURE**

Phosphoric acid solution: Phosphoric acid and water (3:50)

Triethylamine solution: Transfer 3 mL of triethylamine to 1000 mL of water. Adjust with *Phosphoric acid solution* to a pH of 5.3.

Mobile phase: Acetonitrile and *Triethylamine solution* (11:9)

Standard solution: 0.125 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile

Sample solution: Nominally equivalent to 0.125 mg/mL of mycophenolate mofetil prepared as follows. Place Tablets, equivalent to 2.5 g of mycophenolate mofetil based on the label claim, into a 1000-mL volumetric flask. Add 100 mL of water, and shake mechanically for a minimum of 15 min. Add 700 mL of acetonitrile, sonicate for 15 min, and shake mechanically for 20 min. Dilute with acetonitrile to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with acetonitrile to volume. Pass through a nylon filter of 0.45- μ m pore size, and discard the first 5 mL of filtrate.

Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Temperatures

Column: 45°

Autosampler: 10 \pm 5°

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: 94.0%–105.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Times: 5 and 15 min
Standard solution: 0.55 mg/mL of USP Mycophenolate Mofetil RS in *Medium*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.
Detector: UV 304 nm
Path length: 0.1 cm
Blank: *Medium*
Analysis
Samples: *Standard solution* and *Sample solution*
 Calculate the concentration (C_i) of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved at each time point (i):

$$\text{Result} = (A_U/A_S) \times C_S$$

A_U = absorbance from the *Sample solution*
 A_S = absorbance from the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_3)] + (C_1 \times V_3)\} \times (1/L) \times 100$$

C_i = concentration of mycophenolate mofetil in the portion of sample withdrawn at the specified time (mg/mL)
 V = volume of the *Medium*, 900 mL
 L = label claim (mg/Tablet)
 V_3 = volume of the *Sample solution* withdrawn at each time point (mL)
Tolerances: See *Table 1*.

Table 1

Time Point (i)	Time (min)	Tolerances (Q)
1	5	NLT 75%
2	15	NLT 85%

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 900 mL, deaerated
Apparatus 2: 50 rpm
Times: 5 and 15 min
Diluted phosphoric acid: Transfer 5 mL of phosphoric acid to a 25-mL volumetric flask. Dilute with water to volume.
Buffer: 3.0 mL/L of triethylamine in water. Adjust with *Diluted phosphoric acid* to a pH of 5.3.
Mobile phase: Acetonitrile and *Buffer* (45:55)
Diluent: Acetonitrile and *Buffer* (20:80)
Standard stock solution: 0.56 mg/mL of USP Mycophenolate Mofetil RS in *Medium*
Standard solution: 0.11 mg/mL of USP Mycophenolate Mofetil RS in *Diluent*, prepared from the *Standard stock solution*
Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size. Transfer 5 mL of the filtrate to a 25-mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved at each time point (i):

$$\text{Result} = (r_U/r_S) \times C_S \times D$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

D = dilution factor of the *Sample solution*, 5

Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_3)] + (C_1 \times V_3)\} \times (1/L) \times 100$$

C_i = concentration of mycophenolate mofetil in the portion of sample withdrawn at the specified time (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim (mg/Tablet)

V_3 = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (i)	Time (min)	Tolerances (Q)
1	5	NLT 60%
2	15	NLT 80%

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Times: 5 and 15 min

Standard solution: 0.011 mg/mL of USP Mycophenolate Mofetil RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size. Dilute 2 mL of the filtrate with *Medium* to 100 mL.

Detector: UV 250 nm

Path length: 1 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved at each time point (i):

$$\text{Result} = (r_U/r_S) \times C_S \times D$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

D = dilution factor of the *Sample solution*, 50

Calculate the percentage of the labeled amount of mycophenolate mofetil ($C_{23}H_{31}NO_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_s)] + (C_i \times V_s)\} \times (1/L) \times 100$$

C_i = concentration of mycophenolate mofetil in the portion of sample withdrawn at the specified time (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim (mg/Tablet)

V_s = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See Table 3.

Table 3

Time Point (i)	Time (min)	Tolerances (Q)
1	5	NLT 70%
2	15	NLT 85%

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• LIMIT OF DEGRADATION PRODUCTS

Mobile phase, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Sensitivity solution: 0.0625 µg/mL of USP Mycophenolate Mofetil RS in acetonitrile

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis
[NOTE—The run time for the *Sample solution* is 3 times that of the retention time of the mycophenolate mofetil peak.]

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each individual impurity from the *Sample solution*

r_s = peak response of mycophenolate mofetil from the *Standard solution*

C_s = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see Table 4)

Acceptance criteria: See Table 4. [NOTE—Disregard peaks at relative retention times of 1.45 and 2.15. Disregard any peaks less than 0.05%.]

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mycophenolic acid ^a	0.6	1.4	1.0
Mycophenolate N-oxide analog ^b	0.8	1.0	0.2
Mycophenolate mofetil	1.0	—	—
Any single unspecified impurity	—	1.0	0.1
Total degradation products	—	—	1.5

^a (E)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid.

^b 2-Morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate N-oxide.

• LIMIT OF Z-MYCOPHENOLATE MOFETIL

[NOTE—Z-Mycophenolate mofetil is 2-morpholinoethyl (Z)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoate.]

Triethylamine solution: Proceed as directed in the *Assay*.

Mobile phase: Acetonitrile and *Triethylamine solution* (7:13)

Sensitivity solution: 1.25 µg/mL of USP Mycophenolate Mofetil RS in acetonitrile

Standard solution: 0.025 mg/mL of USP Mycophenolate Mofetil RS in acetonitrile

Sample solution: Place Tablets, equivalent to 2.5 g of mycophenolate mofetil based on the label claim, into a 1000-mL volumetric flask. Add 100 mL of water, and shake mechanically for a minimum of 15 min. Add 700 mL of acetonitrile, sonicate for 15 min, and shake mechanically for 20 min. Dilute with acetonitrile to volume. Pass through a nylon filter of 0.45-µm pore size, and discard the first 2 mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Column temperature: 60°

Flow rate: 1.5 mL/min

Run time: 1.7 times the retention time of the mycophenolate mofetil peak

Injection volume: 10 µL

System suitability

Samples: *Sensitivity solution* and *Standard solution*

[NOTE—The relative retention times for mycophenolate mofetil and Z-mycophenolate mofetil are 1.0 and 1.1, respectively.]

Suitability requirements

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of Z-mycophenolate mofetil in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of Z-mycophenolate mofetil from the *Sample solution*

r_s = peak response of mycophenolate mofetil from the *Standard solution*

C_s = concentration of USP Mycophenolate Mofetil RS in the *Standard solution* (mg/mL)

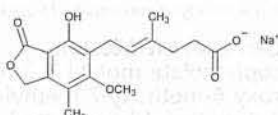
C_u = nominal concentration of mycophenolate mofetil in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.10%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
USP Mycophenolate Mofetil RS

Mycophenolate Sodium



$C_{17}H_{19}NaO_6$ 342.32
4-Hexenoic acid, 6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuran-4-yl)-4-methyl-, monosodium salt, (E)-;
Sodium (E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate [37415-62-6].

DEFINITION

Mycophenolate Sodium contains NLT 98.0% and NMT 102.0% of mycophenolate sodium ($C_{17}H_{19}NaO_6$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197)**
[NOTE—Methods described in *Spectrophotometric Identification Tests, Infrared Absorption* (197K), (197M), or (197A) may be used.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

PROCEDURE

Protect solutions from light.

• **Solution A** (ERR 1-Jun-2016): Acetonitrile, phosphoric acid, and water (100: 0.2: 900)

• **Solution B** (ERR 1-Jun-2016): Acetonitrile, phosphoric acid, and water (800: 0.2: 200)

Mobile phase: See *Table 1*. Return to original conditions, and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
35	10	90
40	10	90

Diluent: Methanol and water (1:9)

Standard solution: 0.08 mg/mL of USP Mycophenolate Sodium RS in *Diluent*. Protect from light.

Sample solution: 0.08 mg/mL of Mycophenolate Sodium in *Diluent*. Protect from light.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 216 nm

Column: 3-mm × 25-cm; 5-μm packing L1

Column temperature: 50°

Flow rate: 0.8 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 0.73%

Tailing factor: 0.7–1.5

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of mycophenolate sodium ($C_{17}H_{19}NaO_6$) in the portion of Mycophenolate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL)

C_U = concentration of Mycophenolate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

ORGANIC IMPURITIES

Protect solutions from light.

Mobile phase, Diluent, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability stock solution 1: 0.02 mg/mL of USP Mycophenolate Mofetil Related Compound B RS prepared as follows. Transfer USP Mycophenolate Mofetil Related Compound B RS to a suitable volumetric flask and dilute in methanol equivalent to 10% of the final volume, and then dilute with water to volume.

System suitability stock solution 2: Transfer 4.0 mL of *System suitability stock solution 1* to a 100-mL volumetric flask and dilute with *Diluent* to volume.

System suitability solution: 0.8 μg/mL of USP Mycophenolate Mofetil Related Compound B RS and 0.08 mg/mL of USP Mycophenolate Sodium RS prepared by dissolving a suitable amount of USP Mycophenolate Sodium RS in *System suitability stock solution 2*

Sensitivity solution: 0.024 μg/mL of USP Mycophenolate Sodium RS in *Diluent* from *Standard solution*

System suitability

Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.5 between the mycophenolate mofetil related compound B and mycophenolate peaks, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Mycophenolate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of mycophenolate from the *Standard solution*

C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL)

C_U = concentration of Mycophenolate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mycophenolate mofetil related compound B ^a	0.9	0.1
Mycophenolate	1.0	—
Any individual unspecified impurity	—	0.07
Total impurities	—	0.4

^a (RS)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3H-isobenzofuranyl-1-one.

SPECIFIC TESTS

• SODIUM CONTENT

Standard stock solution: Use commercially available sodium atomic absorption spectroscopy standard solution of 1000 µg/mL of sodium in 0.5 M nitric acid.

Diluent: Transfer 3 mL of nitric acid to a 250-mL volumetric flask, and dilute with water to volume.

Standard solution A: 6.0 µg/mL of sodium in *Diluent* from *Standard stock solution*

Standard solution B: 9.0 µg/mL of sodium in *Diluent* from *Standard stock solution*

Standard solution C: 12.0 µg/mL of sodium in *Diluent* from *Standard stock solution*

Sample solution: 0.14 mg/mL of Mycophenolate Sodium prepared as follows. Weigh 30–40 mg of Mycophenolate Sodium into a digestion vessel, add 3 mL of nitric acid and digest at 150° for 5 h. Allow to cool, transfer the digestion solution to a 250-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 589.0 nm

Lamp: Sodium hollow-cathode

Flame: Air–acetylene

Blank: *Diluent*

System suitability

Sample: *Standard solution B*

Suitability requirements

Relative standard deviation: NMT 5% for absorbance from three readings

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbances of the *Blank* and *Standard solutions* versus their concentrations of sodium (0, 6.0, 9.0, and 12.0 µg/mL), and draw a calibration curve best fitting the four points. From the graph so obtained, determine the concentration, in µg/mL, of sodium in the *Sample solution*.

Calculate the percentage of sodium in the portion of Mycophenolate Sodium taken:

$$\text{Result} = F \times (C_S/C_U) \times 100$$

F = conversion factor (0.001 mg/µg)

C_S = concentration of sodium in the *Sample solution* (µg/mL)

C_U = concentration of Mycophenolate Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 5.7%–7.7% on the anhydrous basis

- **X-RAY DIFFRACTION** (941): Its X-ray diffraction pattern conforms to that of USP Mycophenolate Sodium RS, similarly determined.

- **WATER DETERMINATION**, *Method 1a* (921): NMT 1.5%
- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial limit does not exceed 10^3 cfu/g. The total yeasts and molds count does not exceed 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from light.
- **USP REFERENCE STANDARDS** (11)
USP Mycophenolate Mofetil Related Compound B RS (RS)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3H-isobenzofuranyl-1-one.
 $C_{17}H_{20}O_6$ 320.34
USP Mycophenolate Sodium RS

Mycophenolic Acid Delayed-Release Tablets

DEFINITION

Mycophenolic Acid Delayed-Release Tablets contain an amount of mycophenolate sodium equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of mycophenolic acid ($C_{17}H_{20}O_6$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Protect solutions from light.

Solution A: Dissolve 21 g of citric acid in a suitable volume of water, add 200 mL of 1 M sodium hydroxide solution, and dilute with water to 1 L.

Buffer: *Solution A* and 0.1 M hydrochloric acid (399:601)

Solution B: Acetonitrile, *Buffer*, and water (40:15:45)

Solution C: Acetonitrile and *Buffer* (85:15)

Mobile phase: See Table 1. Return to original conditions, and re-equilibrate the system.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
9	100	0
18	0	100
22	0	100

Diluent: *Solution B*

Standard solution: 0.385 mg/mL of USP Mycophenolate Sodium RS in *Diluent*. Stir magnetically for at least 60 min to aid dissolution.

Sample stock solution: Nominally equivalent to 9 mg/mL of mycophenolic acid in *Diluent* prepared as follows. Transfer NLT 25 Tablets to a volumetric flask and add *Diluent* to volume. Add a stirring bar and stir vigorously for at least 60 min. Centrifuge a portion of the suspension and use the clear supernatant.

Sample solution: Nominally equivalent to 0.36 mg/mL of mycophenolic acid in *Diluent* from *Sample stock solution*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 251 nm or diode array. [NOTE—Use a diode array detector to perform *Identification test B*.]**Column:** 4.6-mm × 25-cm; 5-μm packing L7**Column temperature:** 40°**Flow rate:** 1 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of mycophenolic acid (C₁₇H₂₀O₆) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of mycophenolate from the *Sample solution* r_S = peak response of mycophenolate from the *Standard solution* C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL) C_U = nominal concentration of mycophenolic acid in the *Sample solution* (mg/mL) M_{r1} = molecular weight of mycophenolic acid, 320.34 M_{r2} = molecular weight of mycophenolate sodium, 342.32**Acceptance criteria:** 95.0%–105.0%**PERFORMANCE TESTS**• **DISSOLUTION (711)**

Protect solutions from light.

Acid stage**Acid stage medium:** 0.1 N hydrochloric acid; 750 mL**Apparatus 2:** 50 rpm**Time:** 2 h**Standard solution:** (L/1000) mg/mL of mycophenolic acid prepared from USP Mycophenolate Sodium RS as follows, where L is the Tablet label claim in mg. Transfer USP Mycophenolate Sodium RS to a suitable volumetric flask, dissolve in methanol equivalent to 5% of the final volume, and dilute with *Acid stage medium* to volume.**Sample solution:** Centrifuge portions of the solution under test or pass through a suitable glass fiber filter of 1-μm pore size.**Instrumental conditions****Mode:** UV**Analytical wavelength:** 250 nm**Cell path length:** Use 0.2 cm for Tablets containing 180 mg of mycophenolic acid per Tablet and 0.1 cm for Tablets containing 360 mg of mycophenolic acid per Tablet.**Blank:** *Acid stage medium***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of mycophenolic acid (C₁₇H₂₀O₆) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL) L = label claim (mg/Tablet) V = volume of *Medium*, 750 mL M_{r1} = molecular weight of mycophenolic acid, 320.34 M_{r2} = molecular weight of mycophenolate sodium, 342.32**Buffer stage****Buffer stage medium:** Replace the volume withdrawal in the *Acid stage* if it is equal to or greater than 2 mL. Add 250 mL of 0.2 M sodium phosphate and adjust, if necessary, to a pH of 6.8 using either 2 M sodium hydroxide or hydrochloric acid; 1000 mL.**Apparatus 2:** 50 rpm**Time:** 1 h. [NOTE—The total time for this analysis is 3 h, where 2 h is from the *Acid stage* and 1 h is from the *Buffer stage*.]**Standard solution:** (L/1000) mg/mL of mycophenolic acid prepared from USP Mycophenolate Sodium RS as follows, where L is the Tablet label claim in mg. Transfer USP Mycophenolate Sodium RS to a suitable volumetric flask, dissolve in methanol equivalent to 5% of the final volume, and dilute with *Buffer stage medium* to volume.**Sample solution:** Centrifuge portions of the solution under test or pass through a suitable glass fiber filter of 1-μm pore size.**Instrumental conditions****Mode:** UV**Analytical wavelength:** 250 nm**Cell path length:** Use 0.2 cm for Tablets containing 180 mg of mycophenolic acid per Tablet and 0.1 cm for Tablets containing 360 mg of mycophenolic acid per Tablet.**Blank:** *Buffer stage medium***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of mycophenolic acid (C₁₇H₂₀O₆) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

 A_U = absorbance of the *Sample solution* A_S = absorbance of the *Standard solution* C_S = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL) L = label claim (mg/Tablet) V = volume of *Medium*, 1000 mL M_{r1} = molecular weight of mycophenolic acid, 320.34 M_{r2} = molecular weight of mycophenolate sodium, 342.32**Tolerances****Acid stage:** NMT 5%**Buffer stage:** NLT 80%

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Weight Variation*

IMPURITIES• **ORGANIC IMPURITIES**

Protect solutions from light.

Mobile phase, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.**System suitability solution:** 0.36 μg/mL of USP Mycophenolate Mofetil Related Compound B RS and 0.385 mg/mL of USP Mycophenolate Sodium RS in *Diluent*. Stir magnetically for at least 60 min to aid dissolution.**Sensitivity solution:** 0.18 μg/mL of USP Mycophenolate Sodium RS in *Diluent* from *Standard solution***System suitability****Samples:** *Standard solution*, *System suitability solution*, and *Sensitivity solution***Suitability requirements****Resolution:** NLT 1.5 between the mycophenolate mofetil related compound B and mycophenolate peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

- r_u = peak response of each individual impurity from the *Sample solution*
 r_s = peak response of mycophenolate from the *Standard solution*
 C_s = concentration of USP Mycophenolate Sodium RS in the *Standard solution* (mg/mL)
 C_u = nominal concentration of mycophenolic acid in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of mycophenolic acid, 320.34
 M_{r2} = molecular weight of mycophenolate sodium, 342.32

Acceptance criteria: See Table 2. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phthalic acid ^a	0.30	—
Impurity 1	0.45	0.1
Phthalic acid monoethyl ester ^{b,a}	0.55	—
Mycophenolate mofetil related compound B ^c	0.90	0.1
Mycophenolate	1.0	—
Ethyl ester of mycophenolate ^d	2.3	0.1
Any individual unspecified impurity	—	0.1
Total impurities	—	0.4

^a Not included in the total impurities.

^b 2-(Ethoxycarbonyl)benzoic acid.

^c (RS)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3H-isobenzofuran-1-one.

^d Ethyl (E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate.

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and protect from moisture. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
 - USP Mycophenolate Mofetil Related Compound B RS (RS)-7-Hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3H-isobenzofuran-1-one.
C₁₇H₂₀O₆ 320.34
 - USP Mycophenolate Sodium RS

Myrrh

DEFINITION

Myrrh is the oleo-gum resin of stems and branches of *Commiphora molmol* Engler and other related species of *Commiphora* other than *Commiphora mukul* (Fam. Burseraceae).

miphora other than *Commiphora mukul* (Fam. Burseraceae).

IDENTIFICATION

A. PROCEDURE

Analysis: Triturate 0.4 g of crushed Myrrh with 1 g of washed sand, shake for a few min with 10 mL of ethyl ether, and filter. Evaporate the filtrate to dryness in a porcelain dish, and add a few drops of nitric acid to the residue.

Acceptance criteria: A purplish violet color is produced instantly.

B. PROCEDURE

Analysis: Transfer 0.1 g of powdered Myrrh to a test tube, and add 1 mL of nitric acid.

Acceptance criteria: A red color is produced. Upon addition of a crystal of vanillin, the red color deepens and does not diminish when water is added.

C. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

Standard solution: 7 µg/mL of (E)-anethole, 8 µg/mL of linalool, and 10 µg/mL each of (–)-bornyl acetate and (R)-(-)-carvone in toluene

Sample solution: 250 mg/mL of finely powdered Myrrh in alcohol. [NOTE—Shake for 1 min, centrifuge, and filter.]

Spray reagent: Dissolve 0.5 mL of *p*-anisaldehyde in 10 mL of glacial acetic acid. Add 85 mL of methanol, and then carefully add 5 mL of sulfuric acid. [NOTE—Prepare fresh immediately before use.]

Application volume: 2 µL for the *Sample solution* and 1 µL for the *Standard solution*

Developing solvent system: Toluene and ethyl acetate (93:7)

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Wash the plate in the *Developing solvent system*, and air-dry before use.]

Spray the plate with *Spray reagent*, heat in an oven at 100° for 5 min, and examine in white light.

Acceptance criteria

The chromatogram of the *Standard solution* exhibits four well-resolved spots: an olive-brown spot due to (E)-anethole at an *R_f* value of about 0.6; an orange-brown spot due to (–)-bornyl acetate at an *R_f* value of about 0.5; a reddish brown spot due to (R)-(-)-carvone at an *R_f* value of about 0.4; and a deep gray spot due to linalool at an *R_f* value of about 0.2.

The chromatogram of the *Sample solution* exhibits an intense purplish red spot at an *R_f* value of about 0.7 and two moderately intense purplish red spots at *R_f* values of about 0.5 and 0.4. The chromatogram of the *Sample solution* may exhibit other spots of varying intensities, including a spot at the origin.

D. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

Standard solution: 4 µg/mL of (E)-anethole and 1 mg/mL of thymol in alcohol

Sample solution: Transfer 0.5 g of finely powdered Myrrh to a test tube containing 5.0 mL of alcohol, and warm the mixture in a water bath for 2–3 min. Cool, and filter.

Developing solvent system: Toluene and ethyl acetate (49:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed. Allow the plate to air-dry, and examine under UV light at 365 nm.

Acceptance criteria: The chromatogram of the *Sample solution* shows no blue-to-violet fluorescent zones in the lower third of the chromatogram (absence of *Commiphora mukul*).

IMPURITIES

- **ARTICLES OF BOTANICAL ORIGIN, Limits of Elemental Impurities (561):** Meets the requirements
- **ARTICLES OF BOTANICAL ORIGIN, Acid-Insoluble Ash (561):** NMT 5.0%
- **ARTICLES OF BOTANICAL ORIGIN, Total Ash (561):** NMT 10.0%
- **ARTICLES OF BOTANICAL ORIGIN, Foreign Organic Matter (561):** NMT 2%

SPECIFIC TESTS

- **BOTANIC CHARACTERISTICS:** Myrrh occurs in rounded or irregular tears, or bumps of agglutinated tears, of variable sizes; brownish yellow to reddish brown, covered with some grayish or yellowish dust, externally; rich brown or reddish brown internally, sometimes marked with white spots or lines; thin splinters, translucent or almost transparent; brittle; waxy, granular, conchoidal fracture; characteristic and aromatic odor; aromatic, bitter, and acid taste.
- **LOSS ON DRYING (731):** Dry 1.0 g of powdered Myrrh between 100° and 105° for 2 h; it loses NMT 15.0% of its weight
- **ARTICLES OF BOTANICAL ORIGIN, Alcohol-Soluble Extractives, Method 2 (561):** 40%–70%
- **ARTICLES OF BOTANICAL ORIGIN, Water-Soluble Extractives, Method 2 (561):** NLT 50%
- **ARTICLES OF BOTANICAL ORIGIN, Volatile Oil Determination (561):** NLT 6.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature, in a dry place.
- **LABELING:** Label it to indicate the species of *Commiphora* from which the oleo-gum resin was obtained. Label it to indicate that it is intended for topical and oropharyngeal use only.

Myrrh Topical Solution

» Prepare Myrrh Topical Solution as follows:

Myrrh	200 g
A mixture of Alcohol and Water (85:15)	900 mL
Alcohol, a sufficient quantity, to make	1000 mL

Macerate about 200 g of coarsely ground Myrrh with an alcohol-water mixture for 48 hours at room temperature in a suitable vessel, which is fitted with a lid and a mechanical stirrer, agitating the mixture with the stirrer. Allow the resulting mixture to stand overnight. Decant the mixture, filter, dilute the filtrate with Alcohol to 1000 mL, and mix.

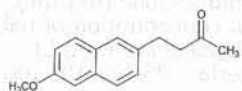
Labeling—Label it to indicate that it is intended for topical and oropharyngeal use only.

Identification—Using Topical Solution as the *Test solution*, proceed as directed for *Identification* tests C and D under *Myrrh*.

Alcohol Determination, Method II (611): between 90.0% and 110.0% of the labeled amount of C_2H_5OH .

Other requirements—It meets the requirements for *Packaging and Storage* and *Labeling* for *Tinctures* under *Botanical Extracts (565)*.

Nabumetone



$C_{15}H_{16}O_2$ 228.29
 2-Butanone, 4-(6-methoxy-2-naphthalenyl)-;
 4-(6-Methoxy-2-naphthyl)-2-butanone [42924-53-8].

DEFINITION

Nabumetone contains NLT 98.0% and NMT 101.0% of nabumetone ($C_{15}H_{16}O_2$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Solution A: Water and glacial acetic acid (999:1), filtered and degassed
Solution B: Acetonitrile and tetrahydrofuran (700:300), filtered and degassed
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
12	60	40
28	20	80
29	60	40
30	60	40

Standard solution: 1.0 mg/mL of USP Nabumetone RS in acetonitrile

Sample solution: 1.0 mg/mL of Nabumetone in acetonitrile

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 4-μm packing L1

Flow rate: 1.3 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nabumetone ($C_{15}H_{16}O_2$) in the portion of Nabumetone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Nabumetone RS in the *Standard solution* (mg/mL)

C_U = concentration of Nabumetone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 0.001% (Official 1-Jan-2018)

ORGANIC IMPURITIES

Solution A, Solution B, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 1 mg/mL of USP

Nabumetone RS and 1 μg/mL of USP Nabumetone Related Compound A RS in acetonitrile

Sample solution: Use the *Sample solution* from the *Assay*.

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times are about 0.9 for nabumetone related compound A and 1.0 for nabumetone; see *Table 2*.]

Suitability requirements

Resolution: NLT 1.5 between nabumetone related compound A and nabumetone

Column efficiency: NLT 3600 theoretical plates

Tailing factor: 0.8–2.0 for the nabumetone peak

Relative standard deviation: NMT 2.0%

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Nabumetone taken:

$$\text{Result} = (F \times r_U) / (r_N + [\Sigma(F \times r_U)]) \times 100$$

F = relative response factor for each impurity (see *Table 2*)

r_U = peak area of each impurity from the *Sample solution*

r_N = peak area of nabumetone from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (w/w, %)
6-Methoxy-2-naphthaldehyde	0.73	0.12	0.1
4-(6'-Methoxy-2'-naphthyl)-butan-2-ol	0.85	0.94	0.1
1-(6'-Methoxy-2'-naphthyl)-but-1-en-3-one (nabumetone related compound A)	0.93	0.25	0.1
Nabumetone	1.0	—	—
5-(6'-Methoxy-2'-naphthyl)-3-methylcyclohex-2-en-1-one	1.2	0.42	0.1
5-(6'-Methoxy-2'-naphthyl)-3-methylcyclohexan-1-one	1.9	1.02	0.1
1,5-Di-(6'-methoxy-2'-naphthyl)-pentan-3-one	2.6	0.91	0.1
6,6-Dimethoxy-2,2'-binaphthyl	2.7	0.10	0.3

Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (w/w, %)
Any individual unknown impurity	—	—	0.1
Total impurities	—	—	0.8

SPECIFIC TESTS• **WATER DETERMINATION, Method 1c (921)**

Sample: 1 g

Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.• **USP REFERENCE STANDARDS (11)**

USP Nabumetone RS

USP Nabumetone Related Compound A RS

1-(6'-Methoxy-2'-naphthyl)-but-1-en-3-one.

C₁₅H₁₄O₂ 226.27**Nabumetone Tablets****DEFINITION**Nabumetone Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of nabumetone (C₁₅H₁₆O₂).**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: Prepare a solution of 6 mL of glacial acetic acid in 350 mL of water. Adjust with 1 N sodium hydroxide to a pH of 3.7, and dilute with water to 400 mL.

Mobile phase: Acetonitrile and *Solution A* (300:200). Filter, and degas.

Diluent: Methanol and water (900:100)

Standard solution: 0.5 mg/mL of USP Nabumetone RS in *Diluent*

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to 500 mg of nabumetone, to a 1000-mL volumetric flask. Add 100 mL of water, and stir with the aid of a magnetic stirrer for 5 min. Dilute with methanol to volume, stir for another 15 min, and filter.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 8-mm × 10-cm; 10-μm packing L1

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitabilitySample: *Standard solution***Suitability requirements**

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of nabumetone (C₁₅H₁₆O₂) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Nabumetone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of nabumetone in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: Sodium lauryl sulfate solution (2 in 100); 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: A known concentration of USP Nabumetone RS in *Medium*

Sample solution: Filter portions of the solution under test, and suitably dilute with *Medium* if necessary.

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the amount of nabumetone (C₁₅H₁₆O₂) dissolved from the differences between the UV absorbances at the wavelengths of maximum and minimum absorbances at about 270 and 296 nm, respectively.

Tolerances: NLT 75% (Q) of the labeled amount of nabumetone (C₁₅H₁₆O₂) is dissolved.

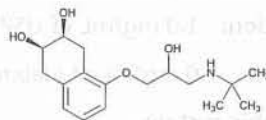
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Nabumetone RS

NadololC₁₇H₂₇NO₄ 309.40

2,3-Naphthalenediol, 5-[3-[(1,1-dimethylethyl)amino]-

2-hydroxypropoxy]-1,2,3,4-tetrahydro-, *cis*-;1-(*tert*-Butylamino)-3-[(5,6,7,8-tetrahydro-*cis*-6,7-dihydroxy-1-naphthyl)oxy]-2-propanol [42200-33-9].**DEFINITION**

Nadolol contains NLT 98.0% and NMT 102.0% of nadolol (C₁₇H₂₇NO₄), calculated on the dried basis.

IDENTIFICATION• **A. INFRARED ABSORPTION (197M)**

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: 0.1% Trifluoroacetic acid

Mobile phase: *Acetonitrile* and *Solution A* (150:850)

Standard solution: 0.2 mg/mL of USP Nadolol RS in *Mobile phase*. Sonication and occasional hand shaking may be necessary for complete dissolution.

Sample solution: 0.2 mg/mL of Nadolol in *Mobile phase*. Sonication and occasional hand shaking may be necessary for complete dissolution.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: NLT 1.7 times the retention time of nadolol peak

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of nadolol (C₁₇H₂₇NO₄) in the portion of Nadolol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of nadolol from the *Sample solution* r_S = peak response of nadolol from the *Standard solution* C_S = concentration of USP Nadolol RS in the *Standard solution* (mg/mL) C_U = concentration of Nadolol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

OTHER COMPONENTS**• RACEMATE COMPOSITION**Sample solution: Prepare a mineral oil dispersion of Nadolol, previously dried, adjusting the thickness of the mull to give an absorbance reading of 0.6 ± 0.1 at a wavelength of 6.3 μm.**Instrumental conditions**

Mode: IR

Wavelength range: 6–9 μm

Analysis: Record the spectrum using mineral oil in the reference beam.

Sample: *Sample solution*

Calculate the percentage of racemate A in the portion of Nadolol taken:

$$\text{Result} = (A_A/A_B) \times (F/A_{av}) \times 100$$

 A_A = uncorrected absorbance at the wavelength of maximum absorbance at about 7.90 μm, corresponding to racemate A A_B = uncorrected absorbance at the wavelength of maximum absorbance at about 8.00 μm, corresponding to racemate B F = fraction of racemate A in a (1:1) reference mixture of racemate A and racemate B, 0.5 A_{av} = average value of the absorbance of (A_A/A_B) in a (1:1) mixture of racemate A and racemate B, 0.9

Acceptance criteria: 40%–60%

IMPURITIES**• RESIDUE ON IGNITION (281):** NMT 0.1%**Delete the following:****• HEAVY METALS, Method II (231):** NMT 30 ppm • (Official 1-

Jan-2018)

• ORGANIC IMPURITIES

Solution A: Proceed as directed in the Assay.

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Acetonitrile (%)
0	85	15
3	85	15
7	50	50
10	50	50
10.5	85	15
15	85	15

Diluent: Acetonitrile and Solution A (15:85)

Standard solution: 0.007 mg/mL of USP Nadolol RS in Diluent. Sonication may be necessary for complete dissolution.

Sensitivity solution: 0.35 μg/mL of USP Nadolol RS in Diluent from *Standard solution*

Sample solution: 0.7 mg/mL of Nadolol in Diluent. Sonication and occasional hand shaking may be necessary for complete dissolution.

Chromatographic system: Proceed as directed in the Assay except for *Injection volume*.

Injection volume: 50 μL

System suitabilitySamples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Relative standard deviation: NMT 2.0%, *Standard solution*Signal-to-noise: NLT 10, *Sensitivity solution***Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Nadolol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_S = peak response of nadolol from the *Standard solution* C_S = concentration of USP Nadolol RS in the *Standard solution* (mg/mL) C_U = concentration of Nadolol in the *Sample solution* (mg/mL) F = relative response factor from Table 2

Acceptance criteria: See Table 2. Disregard peaks below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Nadolol alcohol ^a	0.78	1.35	0.20
Nadolol	1.00	—	—
Nadolol methoxy analog ^b	1.53	1.07	0.20
Nadolol dimer ^c	1.76	1.11	0.20
Diaryl glycerol analog ^d	1.83	1.42	0.20
Naphthyl analog ^e	2.03	3.85	0.20

^a (2*RS*,3*SR*)-5-(2,3-Dihydroxypropoxy)-1,2,3,4-tetrahydronaphthalene-2,3-diol.^b (2*RS*,3*SR*)-5-(2-Hydroxy-3-methoxypropoxy)-1,2,3,4-tetrahydronaphthalene-2,3-diol.^c (2*SR*,3*RS*)-5-[3-*tert*-Butyl(3-[(6*RS*,7*SR*)-6,7-dihydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]oxy)-2-hydroxypropyl)amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydronaphthalene-2,3-diol.^d (2*SR*,3*RS*)-5-(3-[(6*RS*,7*SR*)-6,7-Dihydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]oxy)-2-hydroxypropoxy)-1,2,3,4-tetrahydronaphthalene-2,3-diol and 1,3-Bis[(6*RS*,7*SR*)-6,7-dihydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]oxy]propane-2-ol.^e 1-(*tert*-Butylamino)-3-(naphthalen-1-yl)oxy)propan-2-ol.^f 1-(*tert*-Butylamino)-3-(5,6,7,8-tetrahydronaphthalen-1-yl)oxy)propan-2-ol.

Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dideoxy nadolol ^f	2.10	0.78	0.20
Any unspecified impurity	—	1.00	0.10
Total impurities	—	—	0.50

^a (2*RS*,3*SR*)-5-(2,3-Dihydroxypropoxy)-1,2,3,4-tetrahydronaphthalene-2,3-diol.

^b (2*SR*,3*SR*)-5-(2-Hydroxy-3-methoxypropoxy)-1,2,3,4-tetrahydronaphthalene-2,3-diol.

^c (2*SR*,3*SR*)-5-(3-[(*tert*-Butyl(3-[(6*RS*,7*SR*)-6,7-dihydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]oxy)-2-hydroxypropyl)amino]-2-hydroxypropoxy)-1,2,3,4-tetrahydronaphthalene-2,3-diol.

^d (2*SR*,3*SR*)-5-(3-[(6*RS*,7*SR*)-6,7-dihydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]oxy)-2-hydroxypropoxy)-1,2,3,4-tetrahydronaphthalene-2,3-diol and 1,3-Bis[(6*R*,7*S*)-6,7-dihydroxy-5,6,7,8-tetrahydronaphthalen-1-yloxy]propane-2-ol.

^e 1-(*tert*-Butylamino)-3-(naphthalen-1-yloxy)propan-2-ol.

^f 1-(*tert*-Butylamino)-3-(5,6,7,8-tetrahydronaphthalen-1-yloxy)propan-2-ol.

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry under vacuum at 60° for 3 h.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in well-closed containers.

• USP REFERENCE STANDARDS (11)

USP Nadolol RS

Nadolol Tablets

DEFINITION

Nadolol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of nadolol (C₁₇H₂₇NO₄).

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

Standard solution: 5 mg/mL of USP Nadolol RS in 0.1 N hydrochloric acid

Sample solution: Nominally 5 mg/mL of nadolol from powdered Tablets in 0.1 N hydrochloric acid. Stir for 30 min, using a magnetic stirrer, and place in an ultrasonic bath for an additional 30 min. Centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 100 µL

Developing solvent system: Acetone, chloroform, and 2 N ammonium hydroxide (80:10:10)

Analysis

Samples: Standard solution and Sample solution

Apply the Samples as streaks. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, allow the solvent to evaporate, and examine the chromatogram under short-wavelength UV light.

Acceptance criteria: The R_f value of the principal spot of the Sample solution corresponds to that of the Standard solution.

ASSAY

• PROCEDURE

Mobile phase: A mixture of 700 mL of methanol and 1300 mL of water containing 5.84 g of sodium chloride and 1.0 mL of 0.1 N hydrochloric acid

Standard solution: 0.2 mg/mL of USP Nadolol RS in Mobile phase

Sample solution: Equivalent to 0.2 mg/mL of nadolol from NLT 20 finely powdered Tablets in Mobile phase, prepared as follows. To a suitable amount of the powder in a suitable volumetric flask, add Mobile phase to fill 75% of the total volume. Place the flask in an ultrasonic bath for 15 min, shaking intermittently, and clarify the solution by filtration or centrifugation. Dilute with Mobile phase to volume.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L16

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 3

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of nadolol (C₁₇H₂₇NO₄) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nadolol from the Sample solution

r_S = peak response of nadolol from the Standard solution

C_S = concentration of USP Nadolol RS in the Standard solution (mg/mL)

C_U = nominal concentration of nadolol in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 50 min

Solution A: Dissolve 5.84 g of sodium chloride in 1440 mL of water.

Mobile phase: Methanol and Solution A (560:1440).

Adjust with 0.1 N hydrochloric acid to a pH of 2.5.

Standard solution: USP Nadolol RS in Medium

Sample solution: Use filtered portions of the solution under test. Dilute with Medium, as necessary.

Chromatographic system and System suitability: Proceed as directed in the Assay.

Analysis

Determine the percentage of the labeled amount of nadolol (C₁₇H₂₇NO₄) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times 100$$

r_U = peak response of nadolol from the Sample solution

r_S = peak response of nadolol from the Standard solution

C_S = concentration of USP Nadolol RS in the Standard solution (mg/mL)

L = label claim (mg/tablet)

V = volume of Medium, 900 mL

D = dilution factor used in preparation of the Sample solution

Acceptance criteria: NLT 80% (Q) of the labeled amount of nadolol ($C_{17}H_{27}NO_4$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Nadolol RS

Nadolol and Bendroflumethiazide Tablets

DEFINITION

Nadolol and Bendroflumethiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of nadolol ($C_{17}H_{27}NO_4$) and bendroflumethiazide ($C_{15}H_{14}F_3N_3O_4S_2$).

IDENTIFICATION

- **A.** The retention times of the two major peaks of the *Sample solution* correspond to the corresponding peaks of the *Standard solution*, obtained as directed in the Assay.

ASSAY

PROCEDURE

Use low-actinic glassware for the *Sample solution* and the *Standard solution*.

Mobile phase: Transfer 5.62 g of sodium chloride and 1.97 g of anhydrous sodium acetate to 1000 mL of water in a 2-L volumetric flask. Add 4.0 mL of glacial acetic acid and 800 mL of methanol. Dilute with water to volume.

System suitability solution: 0.4 mg/mL each of USP Nadolol RS and USP 2,4-Disulfamyl-5-trifluoromethylaniline RS in methanol

Standard solution: 0.4 mg/mL of USP Nadolol RS and 0.4 mg/mL of USP Bendroflumethiazide RS in methanol, where *J* is the ratio of the labeled amount of bendroflumethiazide, in mg, to the labeled amount of nadolol, in mg/Tablet.

Sample solution: Nominally equivalent to 0.4 mg/mL of nadolol in methanol prepared as follows. Weigh and finely powder. Transfer a portion of the powder from NLT 20 Tablets, equivalent to 40 mg of nadolol, to a 100-mL volumetric flask. Add methanol, and sonicate for 15 min with occasional shaking. Dilute with methanol to volume, and centrifuge.

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 30-cm; packing L11

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for nadolol and bendroflumethiazide are about 0.3 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.4 between the solvent and 2,4-disulfamyl-5-trifluoromethylaniline peaks, NLT 1.4 between the 2,4-disulfamyl-5-trifluoromethylaniline and nadolol peaks, and NLT 1.7 between the nadolol and bendroflumethiazide peaks; *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nadolol ($C_{17}H_{27}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nadolol from the *Sample solution*

r_S = peak response of nadolol from the *Standard solution*

C_S = concentration of USP Nadolol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of nadolol in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of bendroflumethiazide ($C_{15}H_{14}F_3N_3O_4S_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of bendroflumethiazide from the *Sample solution*

r_S = peak response of bendroflumethiazide from the *Standard solution*

C_S = concentration of USP Bendroflumethiazide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of bendroflumethiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION, Procedure for a Pooled Sample (711)**

Protect solutions from light throughout this test.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: Dissolve the USP Nadolol RS and USP Bendroflumethiazide RS in the minimal amount of methanol, and dilute with *Medium* to the desired concentrations.

Sample solutions: Sample per *Dissolution (711)*.

Using the filtered portion of the solution under test, dilute with *Medium* to a concentration that is similar to the *Standard solution*.

Chromatographic system, System suitability, and Analysis: Proceed as directed in the Assay.

Tolerances: NLT 80% (Q) of the labeled amounts of nadolol ($C_{17}H_{27}NO_4$) and bendroflumethiazide ($C_{15}H_{14}F_3N_3O_4S_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Content Uniformity*

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS (11)**

USP Bendroflumethiazide RS

USP 2,4-Disulfamyl-5-trifluoromethylaniline RS

$C_{15}H_{14}F_3N_3O_4S_2$ 319.29

USP Nadolol RS

Nafcillin Injection

» Nafcillin Injection is a sterile isoosmotic solution of Nafcillin Sodium and one or more buffer substances in Water for Injection. It contains dextrose as a tonicity-adjusting agent. It contains an amount of nafcillin sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of nafcillin

($C_{21}H_{22}N_2O_5S$). It contains no antimicrobial preservatives.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Maintain in the frozen state.

Labeling—It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

USP Reference standards (11)—

USP Endotoxin RS

USP Nafcillin Sodium RS

Identification—The retention time of the major peak for nafcillin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test (85)—It contains not more than 0.13 USP Endotoxin Unit per mg of nafcillin.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

pH (791): between 6.0 and 8.5.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Assay—

Acetic acid solution, 0.05 M Sodium acetate, Diluent, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system—Proceed as directed in the *Assay under Nafcillin Sodium*.

Assay preparation—Allow one container of Injection to thaw, and mix. Transfer an accurately measured volume of Injection, equivalent to about 40 mg of nafcillin, to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay under Nafcillin Sodium*. Calculate the quantity, in mg, of nafcillin ($C_{21}H_{22}N_2O_5S$) in each mL of the Injection taken by the formula:

$$0.1(C/V)(r_U/r_S)$$

in which *C* is the concentration, in μg per mL, of nafcillin in the *Standard preparation*; *V* is the volume, in mL, of Injection taken to prepare the *Assay preparation*; and r_U and r_S are the nafcillin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nafcillin for Injection

» Nafcillin for Injection contains an amount of Nafcillin Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of nafcillin ($C_{21}H_{22}N_2O_5S$).

Change to read:

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

USP Reference standards (11)—

USP Endotoxin RS

USP Nafcillin Sodium RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

Identification—The retention time of the major peak for nafcillin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test (85)—It contains not more than 0.13 USP Endotoxin Unit per mg of nafcillin.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

pH (791): between 6.0 and 8.5, in the solution constituted as directed in the labeling.

Water Determination, Method I (921): between 3.5% and 5.3%.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Other requirements—It meets the requirements for *Uniformity of Dosage Units* (905) and *Labeling* (7), *Labels and Labeling for Injectable Products*.

Assay—

Acetic acid solution, 0.05 M Sodium acetate, Diluent, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system—Proceed as directed in the *Assay under Nafcillin Sodium*.

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Nafcillin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Diluent* to obtain a solution having a concentration of about 0.4 mg of nafcillin ($C_{21}H_{22}N_2O_5S$) per mL.

Assay preparation 2 (where the label states the quantity of nafcillin in a given volume of constituted solution)—Constitute Nafcillin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Diluent* to obtain a solution having a concentration of about 0.4 mg of nafcillin ($C_{21}H_{22}N_2O_5S$) per mL.

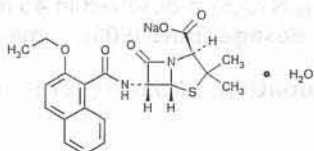
Procedure—Proceed as directed for *Procedure* in the *Assay under Nafcillin Sodium*. Calculate the quantity, in mg, of nafcillin ($C_{21}H_{22}N_2O_5S$) in the portion of constituted Nafcillin for Injection taken by the formula:

$$(C/1000)(L/D)(r_U/r_S)$$

in which *L* is the labeled quantity, in mg, of nafcillin in the portion of Nafcillin for Injection taken; *D* is the concentration, in mg per mL, of nafcillin in *Assay preparation 1* or *Assay preparation 2*, as appropriate, based on the volume of constituted Nafcillin for Injection taken and the extent of dilution; and the other terms are as defined therein.

Perform the above procedure on 10 containers where it is represented as being in a single-dose container and, if necessary, on 10 containers where the label states the quantity of nafcillin in a given volume of constituted solution. Use the individual results to determine the *Uniformity of dosage units* and the average thereof as the *Assay value*.

Nafcillin Sodium



$C_{21}H_{21}N_2NaO_5S \cdot H_2O$ 454.47

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[(2-ethoxy-1-naphthalenyl)carbonyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, monohydrate, [2S-(2 α ,5 α ,6 β)].

Monosodium (2S,5R,6R)-6-(2-ethoxy-1-naphthamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate [7177-50-6].

Anhydrous 436.47 [985-16-0].

» Nafcillin Sodium has a potency equivalent to not less than 820 μ g of nafcillin ($C_{21}H_{22}N_2O_5S$) per mg.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards (11)—

USP Nafcillin Sodium RS

USP Endotoxin RS

Identification—

A: Ultraviolet Absorption (197U)—

Solution: 50 μ g per mL.

Medium: water.

B: The retention time of the major peak for nafcillin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

C: It responds to the tests for *Sodium* (191).

Crystallinity (695): meets the requirements.

pH (791): between 5.0 and 7.0, in a solution containing 30 mg per mL.

Water Determination, Method I (921): between 3.5% and 5.3%.

Other requirements—Where the label states that Nafcillin Sodium is sterile, it meets the requirements for *Sterility Tests* (71) and for *Bacterial endotoxins under Nafcillin for Injection*. Where the label states that Nafcillin Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins under Nafcillin for Injection*.

Assay—

Acetic acid solution—Prepare a 1 in 20 solution of glacial acetic acid and water.

0.05 M Sodium acetate—Dissolve 6.8 g of sodium acetate in about 800 mL of water, adjust with *Acetic acid solution* to a pH of 7.5, dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a suitable filtered and degassed mixture of 0.05 M *Sodium acetate* and acetonitrile (70:30). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

Diluent—Dissolve 6.9 g of sodium citrate in about 800 mL of water, adjust with 1 N hydrochloric acid to a pH of 7.0, dilute with water to 1000 mL, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Nafcillin Sodium RS quantitatively in *Diluent*

to obtain a solution having a known concentration of about 400 μ g of nafcillin ($C_{21}H_{22}N_2O_5S$) per mL.

Resolution solution—Prepare a solution of orcinol in water containing about 35 mg per mL. Add 0.5 mL of this solution to 25 mL of *Standard preparation* to obtain a solution containing about 0.7 mg of orcinol and 400 μ g of nafcillin per mL.

Assay preparation—Transfer about 88 mg of Nafcillin Sodium, accurately weighed, to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm \times 30-cm column containing packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for orcinol and 1.0 for nafcillin; and the resolution between the orcinol and nafcillin peaks is not less than 2.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor for the analyte peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in μ g, of nafcillin ($C_{21}H_{22}N_2O_5S$) in each mg of Nafcillin Sodium taken by the formula:

$$200(C/W)(r_U/r_S)$$

in which C is the concentration, in μ g per mL, of nafcillin ($C_{21}H_{22}N_2O_5S$) in the *Standard preparation*; W is the weight, in mg, of the portion of Nafcillin Sodium taken; and r_U and r_S are the nafcillin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nafcillin Sodium Capsules

» Nafcillin Sodium Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of nafcillin ($C_{21}H_{22}N_2O_5S$).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Nafcillin Sodium RS

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of nafcillin ($C_{21}H_{22}N_2O_5S$) by a suitable validated spectrophotometric analysis of a filtered portion of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Nafcillin Sodium RS in the same medium.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{21}H_{22}N_2O_5S$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Water Determination, Method I (921): not more than 5.0%.

Change to read:

Assay—Proceed as directed under *Antibiotics—Microbial Assays* (81), using not less than 5 Capsules blended for 4 ± 1

minutes in a high-speed glass blender jar containing an accurately measured volume of **Buffer B.1** (CN 1-May-2017). Dilute an accurately measured volume of this stock solution quantitatively with **Buffer B.1** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Nafcillin Sodium for Oral Solution

» Nafcillin Sodium for Oral Solution contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of nafcillin ($C_{21}H_{22}N_2O_5S$). It contains one or more suitable buffers, colors, diluents, dispersants, flavors, and preservatives.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Nafcillin Sodium RS

Uniformity of dosage units (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698): meets the requirements.

pH (791): between 5.5 and 7.5, in the solution constituted as directed in the labeling.

Water Determination, Method I (921): not more than 5.0%.

Change to read:

Assay—Proceed as directed under *Antibiotics—Microbial Assays* (81), using Nafcillin Sodium for Oral Solution constituted as directed in the labeling. Dilute an accurately measured volume of the solution quantitatively with **Buffer B.1** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Nafcillin Sodium Tablets

» Nafcillin Sodium Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of nafcillin ($C_{21}H_{22}N_2O_5S$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Nafcillin Sodium RS

Dissolution (711)—

pH 4.0 buffer—Transfer 10.94 g of anhydrous dibasic sodium phosphate and 12.92 g of citric acid monohydrate to a 1-liter volumetric flask, dissolve in water, dilute with water to volume, and mix.

Medium: pH 4.0 buffer; 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Procedure—Determine the amount of nafcillin ($C_{21}H_{22}N_2O_5S$) dissolved from UV absorbances, at the wavelength of maximum absorbance at about 280 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution

having a known concentration of USP Nafcillin Sodium RS in the same medium.

Tolerances—Not less than 75% (Q) of the labeled amount of nafcillin ($C_{21}H_{22}N_2O_5S$) is dissolved in 45 minutes.

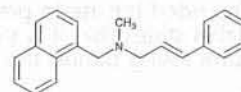
Uniformity of dosage units (905): meet the requirements.

Water Determination, Method I (921): not more than 5.0%.

Change to read:

Assay—Proceed as directed under *Antibiotics—Microbial Assays* (81), using not less than 5 Tablets blended for 4 ± 1 minutes in a high-speed glass blender jar containing an accurately measured volume of **Buffer B.1** (CN 1-May-2017). Dilute an accurately measured volume of this stock solution quantitatively with **Buffer B.1** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Naftifine Hydrochloride



$C_{21}H_{21}N \cdot HCl$ 323.86

1-Naphthalenemethanamine, N-methyl-N-(3-phenyl-2-propenyl)-, hydrochloride, (E)-;
(E)-N-Cinnamyl-N-methyl-1-naphthalenemethylamine hydrochloride [65473-14-5].

DEFINITION

Naftifine Hydrochloride contains NLT 99.0% and NMT 101.0% of naftifine hydrochloride ($C_{21}H_{21}N \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Mobile phase: *n*-Hexane, absolute alcohol, dimethylformamide, and formic acid (200:60:40:2). Cover tightly with a moisture-proof film, and allow to stand for 12 h at room temperature.

Standard solution: 0.2 mg/mL of USP Naftifine Hydrochloride RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Naftifine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L3

Flow rate: 2 mL/min

Injection volume: 15 μ L

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of naftifine hydrochloride ($C_{21}H_{21}N \cdot HCl$) in the portion of Naftifine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

- r_s = peak response from the *Standard solution*
 C_s = concentration of USP Naftifine Hydrochloride RS in the *Standard solution* (mg/mL)
 C_U = concentration of the *Sample solution* (mg/mL)
 Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): 10 ppm • (Official 1-Jan-2018)

ORGANIC IMPURITIES

Mobile phase, *Standard solution*, *Sample solution*, and *Chromatographic system*: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Naftifine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

- r_U = peak response for each impurity
 r_T = sum of all the peak responses

Acceptance criteria

Individual impurity: NMT 0.1%

Total impurities: NMT 1.0%

SPECIFIC TESTS

- **LOSS ON DRYING** (731)

Analysis: Dry over phosphorus pentoxide at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)
USP Naftifine Hydrochloride RS

Naftifine Hydrochloride Cream**DEFINITION**

Naftifine Hydrochloride Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of naftifine hydrochloride ($C_{21}H_{21}N \cdot HCl$) in a water-miscible base.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**PROCEDURE**

Mobile phase: *n*-Hexane, alcohol, dimethylformamide, and formic acid (200:60:40:2). Cover tightly with a moisture-proof film, and allow to stand for 12 h at room temperature.

Standard solution: 0.2 mg/mL of USP Naftifine Hydrochloride RS in *Mobile phase*

Sample solution: Transfer 1000 mg of Cream to a 100-mL volumetric flask. Dissolve in 60 mL of methanol, mix vigorously for 2 min, and dilute with methanol to volume. Heat at 45° for 5 min, and cool to room temperature.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 5-μm packing L3

Flow rate: 2 mL/min

Injection volume: 15 μL

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naftifine hydrochloride ($C_{21}H_{21}N \cdot HCl$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_s) \times (C_s/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_s = peak response from the *Standard solution*
 C_s = concentration of USP Naftifine Hydrochloride RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of naftifine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **MINIMUM FILL** (755): Meets the requirements

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED ORGANISMS** (62): It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **pH** (791): 4.0–6.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)
USP Naftifine Hydrochloride RS

Naftifine Hydrochloride Gel**DEFINITION**

Naftifine Hydrochloride Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of naftifine hydrochloride ($C_{21}H_{21}N \cdot HCl$) in a water-miscible base.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**PROCEDURE**

Mobile phase: *n*-Hexane, alcohol, dimethylformamide, and formic acid (200:60:40:2). Cover tightly with a moisture-proof film, and allow to stand for 12 h at room temperature.

Standard solution: 0.2 mg/mL of USP Naftifine Hydrochloride RS in *Mobile phase*

Sample solution: Transfer 1000 mg of Gel to a 100-mL volumetric flask. Dissolve in 60 mL of methanol, mix vigorously for 2 min, and dilute with methanol to volume. Heat at 45° for 5 min, and cool to room temperature.

Chromatographic system(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; 5-μm packing L3

Flow rate: 2 mL/min

Injection volume: 20 μL

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of naftifine hydrochloride (C₂₁H₂₁N · HCl) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Naftifine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = nominal concentration of naftifine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **MINIMUM FILL** ⟨755⟩: Meets the requirements

SPECIFIC TESTS

- **CONTENT OF ALCOHOL**

Internal standard solution: Transfer 10.0 mL of *n*-propyl alcohol to a 200-mL volumetric flask, and dilute with water to volume.*Standard stock solution*: 10.0 mg/mL of alcohol*Standard solution*: Transfer 3.0 mL of *Internal standard solution* to a 10-mL volumetric flask, and dilute with *Standard stock solution* to volume.*Sample solution*: Transfer 250 mg of Gel to a suitable container. Add 14.0 mL of water and 6.0 mL of *Internal standard solution*, and shake for 15 min.**Chromatographic system**(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 3.2-mm × 1.5-m; 80- to 100-mesh support S3

Temperatures

Injector: 200°

Column: 170°

Detector: 200°

Carrier gas: Nitrogen

Flow rate: 45 mL/min

Injection volume: 1 μL

System suitabilitySample: *Standard solution***Suitability requirements**

Resolution: NLT 2.0 between alcohol and the internal standard

Capacity factor, K' : 2.0–3.5 for alcohol and 6.0–8.0 for the internal standard

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.5%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of alcohol (C₂H₅OH) in the portion of Gel taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

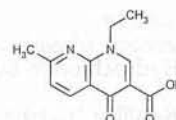
 R_U = peak response ratio of alcohol to the internal standard from the *Sample solution* R_S = peak response ratio of alcohol to the internal standard from the *Standard solution* C_S = concentration of alcohol in the *Standard solution* (mg/mL) C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 40%–45%

- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED ORGANISMS** ⟨62⟩: It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **PH** ⟨791⟩: 5.5–7.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Naftifine Hydrochloride RS

Nalidixic Acid

C₁₂H₁₂N₂O₃ 232.24
 1,8-Naphthyridine-3-carboxylic acid, 1-ethyl-1,4-dihydro-7-methyl-4-oxo-;
 1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid [389-08-2].

DEFINITIONNalidixic Acid contains NLT 99.0% and NMT 101.0% of nalidixic acid (C₁₂H₁₂N₂O₃), calculated on the dried basis.**IDENTIFICATION**

- **A. INFRARED ABSORPTION** ⟨197K⟩
- **B. ULTRAVIOLET ABSORPTION** ⟨197U⟩
Analytical wavelength: 258 nm
Sample solution: 5 μg/mL in 0.01 N sodium hydroxide
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY

- **PROCEDURE**

Sample solution: Dissolve 250 mg of Nalidixic Acid in 30 mL of dimethylformamide previously neutralized with thymolphthalein TS.**Titrimetric system**

Mode: Direct titration

Titrant: 0.1 N lithium methoxide VS in methanol

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with *Titrant*, using a magnetic stirrer and taking precautions against absorption of atmospheric carbon dioxide. Each mL of 0.1 N lithium methoxide is equivalent to 23.22 mg of nalidixic acid (C₁₂H₁₂N₂O₃).

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩: NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** ⟨231⟩: NMT 20 ppm • (Official 1-

(Jan-2018)

- **ORGANIC IMPURITIES**

Standard stock solution: 1.0 mg/mL of USP Nalidixic Acid RS in chloroform*Standard solutions*: Dilute the *Standard stock solution* quantitatively with chloroform to obtain *Standard solutions* having the compositions shown in Table 1.

Table 1

Standard solution	Dilution	Concentration (mg/mL)	Percentage (for comparison with Sample solution)
A	1 in 10	0.1	0.5
B	1 in 25	0.04	0.2
C	1 in 50	0.02	0.1

Sample solution: 20 mg/mL of Nalidixic Acid in chloroform

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: Alcohol, chloroform, and 5 M ammonium hydroxide (70:20:10)

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Apply the *Samples* separately, position the plate in a chromatographic chamber, and develop the chromatograms until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate with the aid of warm circulating air. Examine the plate under short-wavelength UV light. Compare the intensities of any secondary spots observed in the *Sample solution* with those of the principal spots in the *Standard solutions*.

Acceptance criteria: No secondary spot is more intense than the principal spot obtained from *Standard solution A* (0.5%), and the sum of the intensities of all secondary spots obtained from the *Sample solution* is NMT 1.0%.

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** (741): 225°–231°

• **LOSS ON DRYING** (731)

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Nalidixic Acid RS

Nalidixic Acid Oral Suspension

DEFINITION

Nalidixic Acid Oral Suspension contains NLT 95.0% and NMT 105.0% of the labeled amount of nalidixic acid ($C_{12}H_{12}N_2O_3$) in a suitable aqueous vehicle.

IDENTIFICATION

• **A.** The retention time of the nalidixic acid peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Solution A: 2.41 g/L of dibasic potassium phosphate in water

Solution B: 7.49 g/L of hexadecyltrimethylammonium bromide in methanol

Mobile phase: Methanol, *Solution A*, and *Solution B* (325:325:350), with an apparent pH of about 10.

Internal standard solution: 0.8 mg/mL of sulfanilic acid in *Mobile phase*

Standard stock solution: 0.18 mg/mL of USP Nalidixic Acid RS in methanol

Standard solution: 36 μ g/mL of USP Nalidixic Acid RS prepared as follows. Transfer 5.0 mL of *Standard stock solution* and 1.0 mL of *Internal standard solution* to a 25-mL volumetric flask. Dilute with methanol to volume.

Sample stock solution: Nominally 0.3 mg/mL of nalidixic acid prepared as follows. Transfer 150 mg of nalidixic acid from a volume of freshly mixed Oral Suspension to a 500-mL volumetric flask. Add 400 mL of methanol, and sonicate for 30 min. Shake by mechanical means for 30 min, sonicate again for 30 min, dilute with methanol to volume, mix, and filter.

Sample solution: Nominally 36 μ g/mL of nalidixic acid prepared as follows. Transfer 3.0 mL of the clear filtrate from *Sample stock solution* and 1.0 mL of *Internal standard solution* to a 25-mL volumetric flask, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for sulfanilic acid and nalidixic acid are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1 between sulfanilic acid and nalidixic acid

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nalidixic acid ($C_{12}H_{12}N_2O_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of nalidixic acid to sulfanilic acid from the *Sample solution*

R_S = peak area ratio of nalidixic acid to sulfanilic acid from the *Standard solution*

C_S = concentration of USP Nalidixic Acid RS in the *Standard solution* (μ g/mL)

C_U = nominal concentration of nalidixic acid in the *Sample solution* (μ g/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements for Oral Suspension packaged in single-unit containers

• **DELIVERABLE VOLUME** (698): Meets the requirements for Oral Suspension packaged in multiple-unit containers

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Nalidixic Acid RS

Nalidixic Acid Tablets

DEFINITION

Nalidixic Acid Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of nalidixic acid ($C_{12}H_{12}N_2O_3$).

IDENTIFICATION

- **A.** The retention time of the nalidixic acid peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: 2.41 g/L of dibasic potassium phosphate in water

Solution B: 7.49 g/L of hexadecyltrimethylammonium bromide in methanol

Mobile phase: Methanol, *Solution A*, and *Solution B* (325:325:350), with an apparent pH of about 10

Internal standard solution: 0.8 mg/mL of sulfanilic acid in *Mobile phase*

Standard stock solution: 0.18 mg/mL of USP Nalidixic Acid RS in methanol

Standard solution: 36 µg/mL of USP Nalidixic Acid RS prepared as follows. Transfer 5.0 mL of *Standard stock solution* and 1.0 mL of *Internal standard solution* to a 25-mL volumetric flask. Dilute with methanol to volume.

Sample stock solution: Nominally 0.3 mg/mL of nalidixic acid prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer 150 mg of nalidixic acid from a portion of the powder to a 500-mL volumetric flask. Add 400 mL of methanol, and sonicate for 30 min. Shake by mechanical means for 30 min, sonicate again for 30 min, dilute with methanol to volume, mix, and filter.

Sample solution: Nominally 36 µg/mL of nalidixic acid prepared as follows. Transfer 3.0 mL of the clear filtrate from *Sample stock solution* and 1.0 mL of *Internal standard solution* to a 25-mL volumetric flask, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for sulfanilic acid and nalidixic acid are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1 between sulfanilic acid and nalidixic acid

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of nalidixic acid (C₁₂H₁₂N₂O₃) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of nalidixic acid to sulfanilic acid from the *Sample solution*

R_S = peak area ratio of nalidixic acid to sulfanilic acid from the *Standard solution*

C_S = concentration of USP Nalidixic Acid RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of nalidixic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: pH 8.60 buffer prepared by mixing 2.3 volumes of 0.2 M sodium hydroxide with 2.5 volumes of 0.2 M monobasic potassium phosphate and 2.0 volumes of methanol. Cool, mix with water to obtain 10 volumes of solution, and adjust, if necessary, by

adding 1 N sodium hydroxide to a pH of 8.60 ± 0.05; 900 mL.

Apparatus 2: 60 rpm

Time: 30 min

Standard solution: A known concentration of USP Nalidixic Acid RS in 0.01 N sodium hydroxide

Sample solution: A filtered solution under test, suitably diluted with 0.01 N sodium hydroxide, if necessary, in comparison with *Standard solution*

Blank: Mixture of *Medium* and 0.01 N sodium hydroxide in the same proportions as present in the *Sample solution*

Instrumental conditions

Mode: UV

Analytical wavelength: 258 nm

Analysis: Determine the amount of nalidixic acid (C₁₂H₁₂N₂O₃) dissolved.

Tolerances: NLT 80% (Q) of the labeled amount of nalidixic acid (C₁₂H₁₂N₂O₃) is dissolved.

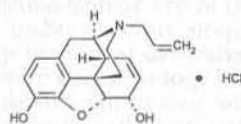
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS (11)**

USP Nalidixic Acid RS

Nalorphine Hydrochloride

C₁₉H₂₁NO₃ · HCl 347.84

Morphinan-3,6-diol, 7,8-didehydro-4,5-epoxy-17-(2-propenyl)-(5α,6α)-, hydrochloride.

17-Allyl-7,8-didehydro-4,5α-epoxymorphinan-3,6α-diol hydrochloride [57-29-4].

» Nalorphine Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of C₁₉H₂₁NO₃ · HCl, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards (11)—

USP Nalorphine Hydrochloride RS

Identification—

A: *Infrared Absorption* (197K).

B: *Ultraviolet Absorption* (197U)—

Solution: 100 µg per mL.

Medium: water.

C: A solution of it responds to the tests for *Chloride* (191).

Specific rotation (781S): between −122° and −125°.

Test solution: 20 mg per mL, in water.

Loss on drying (731)—Dry it in vacuum at 100° for 2 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.1%.

Assay—Transfer about 25 mg of Nalorphine Hydrochloride, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Concomitantly determine the absorbances of this solution and of a *Standard solution* of USP Nalorphine Hydrochloride RS in the

same medium having a known concentration of about 100 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 285 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{19}H_{21}NO_3 \cdot HCl$ in the Nalorphine Hydrochloride taken by the formula:

$$0.25C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Nalorphine Hydrochloride RS in the Standard solution; and A_U and A_S are the absorbances of the solution of Nalorphine Hydrochloride and the Standard solution, respectively.

Nalorphine Hydrochloride Injection

» Nalorphine Hydrochloride Injection is a suitably buffered, sterile solution of Nalorphine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nalorphine hydrochloride ($C_{19}H_{21}NO_3 \cdot HCl$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards (11)—

USP Endotoxin RS

USP Nalorphine Hydrochloride RS

Identification—Apply 15 µL of Injection and 15 µL of a Standard solution of USP Nalorphine Hydrochloride RS in methanol containing 5 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the applications to dry, and develop the chromatogram in an equilibrated chamber containing methanol until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Observe the plate under short- and long-wavelength UV light: the R_f value of the principal spot obtained from the Injection corresponds to that obtained from the Standard solution.

Bacterial Endotoxins Test (85)—It contains not more than 11.6 USP Endotoxin Units per mg of nalorphine hydrochloride.

pH (791): between 6.0 and 7.5.

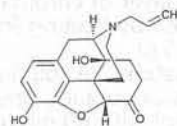
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of nalorphine hydrochloride, to a 25-mL centrifuge separator, add 1 mL of 3 N hydrochloric acid, and dilute with water to about 10 mL. Extract with five 5-mL portions of chloroform, separating the layers by centrifugation before drawing off each chloroform extract, and discard the chloroform extracts. Transfer the aqueous layer to a 100-mL volumetric flask with the aid of water, dilute with water to volume, and mix. Proceed as directed in the Assay under *Nalorphine Hydrochloride*, beginning with "Concomitantly determine the absorbances." Calculate the quantity, in mg, of $C_{19}H_{21}NO_3 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(0.1C / V)(A_U / A_S)$$

in which V is the volume, in mL, of Injection taken, and C , A_U , and A_S are as defined therein.

Naloxone Hydrochloride



$C_{19}H_{21}NO_4 \cdot HCl$ 363.84
Morphinan-6-one, 4,5-epoxy-3,14-dihydroxy-17-(2-propenyl)-, hydrochloride, (5α)-;
17-Allyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride [357-08-4].
Dihydrate 399.87
[51481-60-8].

DEFINITION

Naloxone Hydrochloride is anhydrous or contains two molecules of water of hydration. It contains NLT 98.0% and NMT 100.5% of naloxone hydrochloride ($C_{19}H_{21}NO_4 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

Sample: About 150 mg

Analysis: Dissolve the *Sample* in 25 mL of water in a small separator, and add a few drops of 6 N ammonium hydroxide slowly until no more white precipitate is formed. Extract with three 5-mL portions of chloroform, and pass the extracts through a dry filter, collecting the filtrate in a small flask. Evaporate the filtrate on a steam bath to dryness, and dry the residue at 105° for 1 h.

Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Sample: About 300 mg

Analysis: Dissolve the *Sample*, previously dried, in a mixture of 40 mL of glacial acetic acid and 10 mL of acetic anhydride. Add 10 mL of mercuric acetate TS and 1 drop of methyl violet TS. Titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 36.38 mg of naloxone hydrochloride ($C_{19}H_{21}NO_4 \cdot HCl$).

Acceptance criteria: 98.0%–100.5% on the dried basis

OTHER COMPONENTS

• CONTENT OF CHLORIDE

Sample: About 300 mg

Analysis: Dissolve the *Sample* in 50 mL of methanol contained in a 125-mL conical flask, and add 5 mL of glacial acetic acid and 2 drops of eosin Y TS. Titrate with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.

Acceptance criteria: 9.54%–9.94% on the dried basis

IMPURITIES

• NOROXYMORPHONE HYDROCHLORIDE [(–)-4,5α-EPOXY-3,14-DIHYDROXYMORPHINAN-6-ONE HYDROCHLORIDE] AND OTHER IMPURITIES

Standard solution A: 7.6 mg/mL of USP Naloxone RS in chloroform

Standard solution B: 0.084 mg/mL of USP Noroxymorphone Hydrochloride RS in dilute methanol (3 in 5)

Sample solution: Transfer about 40 mg to a 5-mL volumetric flask. Dissolve completely in 2.0 mL of water, add methanol to volume, and mix.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel, previously activated by heating for 15 min at 105°

Application volume: 5 µL

Developing solvent system: A solution (1 in 20) of methanol in ammoniacal butanol, prepared by shaking 100 mL of butyl alcohol with 60 mL of ammonium hydroxide solution (1 in 100) and discarding the lower layer

Spray reagent: 5 mg/mL of potassium ferricyanide in ferric chloride solution (1 in 10). Prepare immediately before use.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram, protected from light, until the solvent front has moved about 10 cm from the point of application. Remove the plate, dry thoroughly, and spray with *Spray reagent*.

Acceptance criteria: Other than the principal spot corresponding in R_f value to that of USP Naloxone RS and the spot at the origin (ammonium chloride), no other spot is more intense than the spot corresponding to that of USP Noroxymorphone Hydrochloride RS (1.0%).

SPECIFIC TESTS• **OPTICAL ROTATION**, *Specific Rotation* (781S)

Sample solution: 25 mg/mL in water

Acceptance criteria: −170° to −181°

• **LOSS ON DRYING** (731)

Analysis: Dry at 105° to constant weight.

Acceptance criteria: NMT 0.5% for the anhydrous form and NMT 11.0% for the hydrous form

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

• **USP REFERENCE STANDARDS** (11)

USP Naloxone RS

USP Noroxymorphone Hydrochloride RS

Naloxone Hydrochloride Injection

» Naloxone Hydrochloride Injection is a sterile, isotonic solution of Naloxone Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of naloxone hydrochloride ($C_{19}H_{21}NO_4 \cdot HCl$). It may contain suitable preservatives.

Packaging and storage—Preserve in single-dose or in multiple-dose containers of Type I glass, protected from light.

USP Reference standards (11)—

USP Endotoxin RS

USP Naloxone RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

Bacterial Endotoxins Test (85)—It contains not more than 500 USP Endotoxin Units per mg of Naloxone Hydrochloride.

pH (791): between 3.0 and 6.5.

Limit of 2,2'-binaloxone—

Mobile phase, Diluting solvent, System suitability preparation, and Chromatographic system—Prepare as directed in the *Assay*.

Ferric chloride solution—Transfer 4 mL of ferric chloride TS to a 100-mL volumetric flask, dilute with water to volume, and mix.

Identification solution—Dissolve 10 mg of naloxone in 100 mL of 0.1 N hydrochloric acid. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and add 0.5 mL of *Ferric chloride solution*. Heat on a steam bath for 10 minutes, cool, dilute with water to volume, and mix.

Standard solution—Transfer 2.0 mL of the *Standard preparation* prepared as directed in the *Assay* to a 100-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix.

Test solution—Use the *Assay preparation*.

Procedure—Separately inject equal volumes (about 100 µL) of the *Identification solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the peak responses for naloxone and 2,2'-binaloxone. The relative retention times are about 2.8 for the naloxone dimer and 1.0 for naloxone. Calculate the percentage of 2,2'-binaloxone in the volume of Injection taken by the formula:

$$(100 / L)(363.84 / 327.38)(C / 1.8)(V_b / V)(r_u / r_s)$$

in which L is the labeled quantity, in µg per mL, of naloxone hydrochloride ($C_{19}H_{21}NO_4 \cdot HCl$) in the Injection taken, 363.84 and 327.38 are the molecular weights of anhydrous naloxone hydrochloride and naloxone, respectively, C is the concentration, in µg per mL, of USP Naloxone RS in the *Standard solution*, 1.8 is the ratio of UV absorptivity of 2,2'-binaloxone to that of naloxone hydrochloride, V_b is the volume, in mL, of the *Test solution*, V is the volume, in mL, of Injection taken, r_u is the peak response for 2,2'-binaloxone obtained from the *Test solution*, and r_s is the peak response for naloxone obtained from the *Standard solution*. Not more than 4.0% is found.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 1.36 g of sodium 1-octanesulfonate, 1.0 g of sodium chloride, 580 mL of water, 420 mL of methanol, and 1.0 mL of phosphoric acid. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

Diluting solvent—Transfer 150 mg of edetate disodium to a 2000-mL volumetric flask, and add 0.9 mL of hydrochloric acid. Dilute with water to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Naloxone RS in *Diluting solvent*, and dilute quantitatively, and stepwise if necessary, with *Diluting solvent* to obtain a solution having a known concentration of about 10 µg per mL.

Assay preparation 1 (for Injection labeled to contain not more than 100 µg of naloxone hydrochloride per mL)—Transfer an accurately measured volume of Injection, equivalent to about 100 µg of naloxone hydrochloride, to a 10-mL volumetric flask, add *Diluting solvent* to volume, and mix.

Assay preparation 2 (for Injection labeled to contain more than 100 µg of naloxone hydrochloride per mL)—Transfer an accurately measured volume of Injection, equivalent to about 2000 µg of naloxone hydrochloride, to a 200-mL volumetric flask, add *Diluting solvent* to volume, and mix.

System suitability preparation—Prepare a solution in *Diluting solvent* containing about 20 µg of USP Naloxone RS and about 2.5 µg of acetaminophen per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 229-nm detector

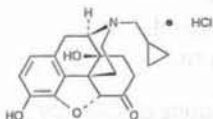
and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation* (about 100 µL) and the *System suitability preparation* (about 20 µL), and record the peak responses as directed under *Procedure*: the resolution, R , between the acetaminophen and naloxone peaks is not less than 8, and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 1.5%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the appropriate *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for acetaminophen and 1.0 for naloxone. Calculate the quantity, in µg, of $C_{19}H_{21}NO_4 \cdot HCl$ in each mL of the injection taken by the formula:

$$(363.84 / 327.38)V_a(C / V)(r_U / r_S)$$

in which 363.84 and 327.38 are the molecular weights of anhydrous naloxone hydrochloride and naloxone, respectively; V_a is the volume, in mL, of the *Assay preparation*; C is the concentration, in µg per mL, of USP Naloxone RS in the *Standard preparation*; V is the volume, in mL, of injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Naltrexone Hydrochloride



$C_{20}H_{23}NO_4 \cdot HCl$ 377.86

Morphinan-6-one, 17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxy-, hydrochloride, (5 α)-

17-(Cyclopropylmethyl)-4,5 α -epoxy-3,14-dihydroxymorphinan-6-one hydrochloride [16676-29-2].

» Naltrexone Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{20}H_{23}NO_4 \cdot HCl$, calculated on the anhydrous, solvent-free basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Naltrexone RS

USP Naltrexone Related Compound A RS

N-(3-Butenyl)-noroxymorphone hydrochloride.

$C_{20}H_{23}NO_4 \cdot HCl$ 377.87

Completeness of solution (641)—A 650-mg portion dissolves in 10 mL of water to yield a clear solution.

Identification, Infrared Absorption (197K)—

Test specimen—Dissolve about 150 mg in 25 mL of water in a small separator, add a few drops of 6 N ammonium hydroxide slowly until no more white precipitate is formed. Extract with three 5-mL portions of chloroform, filter the extracts through a dry filter, collecting the filtrate in a small flask. Evaporate the filtrate on a steam bath to dryness, and dry the residue at 105° for one hour.

Specific rotation (781S): between −187° and −197°, calculated on the anhydrous, solvent-free basis.

Test solution: 25 mg per mL, in water.

Water Determination, Method I (921)—Determine the water content as directed. [NOTE—The result of this test is used in the calculation of *Limit of total solvents*.]

Residue on ignition (281): not more than 0.1%.

Delete the following:

Heavy metals, Method II (231): not more than 0.002%. • (Official 1-Jan-2018)

Limit of total solvents—

Internal standard stock solution—Transfer 6.0 mL of isopropyl alcohol to a 500-mL volumetric flask, dilute with water to volume, and mix. [NOTE—The isopropyl alcohol must be free of alcohol impurities.]

Internal standard solution—Transfer 5.0 mL of the *Internal standard stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard solution—Prepare a solution of methanol and alcohol (C_2H_5OH) in water to obtain a solution having a known concentration of about 16 mg of each per mL. Transfer 3.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Test solution—Transfer about 75 mg of Naltrexone Hydrochloride, accurately weighed, to a suitable container, add 5.0 mL of *Internal standard solution*, and shake to dissolve.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm × 1.8-m glass column packed with 80- to 100-mesh support S3. The column temperature is maintained at 150°, and the injection port and detector temperatures are maintained at 170°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.24 for methanol, 0.53 for alcohol, and 1.0 for isopropyl alcohol.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard solution* and the *Test solution* into the gas chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentages of methanol and alcohol in the portion of Naltrexone Hydrochloride taken by the formula:

$$100(C_S / C_U)(R_U / R_S)$$

in which C_S is the concentration, in mg per mL, of methanol or alcohol (C_2H_5OH) in the *Standard solution*; C_U is the concentration, in mg per mL, of Naltrexone Hydrochloride in the *Test solution*; and R_U and R_S are the peak response ratios of methanol or alcohol to isopropyl alcohol obtained from the *Test solution* and the *Standard solution*, respectively. To the sum of the percentages of methanol and alcohol, add the percentage of water as determined in the test for *Water*: the sum of water and alcoholic solvents is not more than 5.0% for the anhydrous form, and not more than 11.0% for the dihydrate form.

Change to read:

Related compounds—Proceed as directed in the *Assay*. From the chromatogram of the *Assay preparation*, calculate the percentage of each related compound in Naltrexone Hydrochloride taken by the formula:

$$100F(C/W)(r_U/r_S) \bullet \text{ (ERR 1-Jun-2016)}$$

in which F is the relative response factor for each impurity; C is the concentration, in mg per mL, of USP Naltrexone RS in the *Standard preparation*; W is the weight, in mg, of Naltrexone Hydrochloride taken for the *Assay preparation*; r_U is the peak response of the relevant related compound ob-

tained from the *Assay preparation*; and r_s is peak response of naltrexone obtained from the *Standard preparation*. [NOTE—The relative response factor is 0.43 for 2,2'-bisnaltrexone, 0.25 for 10-ketonaltrexone, and 1.0 for all other related compound peaks.] Not more than 0.5% of any individual related compound is found, and the total of all related compounds is not more than 1.5%.

Content of chloride—Transfer about 300 mg, accurately weighed, to a 250-mL conical flask, add 50 mL of methanol, 50 mL of water, and 3 mL of nitric acid, and mix to dissolve. Titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride: between 9.20% and 9.58%, calculated on the anhydrous, solvent-free basis is found.

Assay—

Solution A—Dissolve about 1.08 g of sodium 1-octanesulfonate and about 23.8 g of sodium acetate in 800 mL of water. Add 1.0 mL of triethylamine and 200 mL of methanol, and mix. Adjust with glacial acetic acid to a pH of 6.5 \pm 0.1. Filter and degas prior to use.

Solution B—Dissolve about 1.08 g sodium 1-octanesulfonate and about 23.8 g sodium acetate in 400 mL of water. Add 1.0 mL triethylamine and 600 mL of methanol, and mix. Adjust with glacial acetic acid to a pH of 6.5 \pm 0.1. Filter and degas prior to use.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*.

Standard preparation—Transfer an accurately weighed quantity of about 22.5 mg of USP Naltrexone RS to a 10-mL volumetric flask. Add 1.5 mL of methanol and 0.6 mL of 0.1 N hydrochloric acid. Dissolve by swirling the flask, and dilute with 0.1 M phosphoric acid to volume.

Resolution solution—Transfer about 3.0 mg, accurately weighed, of USP Naltrexone Related Compound A RS to a 10-mL volumetric flask. Add 3.0 mL of methanol, and dissolve by swirling. Dilute with 0.1 M phosphoric acid to volume, and mix. Transfer 0.5 mL of this solution to a 10-mL volumetric flask, add 5.0 mL of *Standard preparation*, dilute with 0.1 M phosphoric acid to volume, and mix.

Assay preparation—Transfer an accurately weighed quantity of about 25 mg of Naltrexone Hydrochloride to a 10-mL volumetric flask. Dissolve in and dilute with 0.1 M phosphoric acid to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm \times 15-cm column that contains packing L1 and is programmed to provide, at a flow rate of about 1 mL per minute, a variable mixture of *Solution A* and *Solution B*. At the time the specimen is injected into the chromatograph, the percentage of *Solution A* is 100%; over the next 35 minutes, the proportion of *Solution B* is increased linearly to 100%, and then over the next minute, decreased linearly to 100% of *Solution A*. Allow the system to equilibrate until the late eluting peak has been observed, approximately 17 minutes later. Chromatograph about 20 μ L of the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.55 for noroxymorphone, 0.70 for 10-hydroxynaltrexone, 1.0 for naltrexone, 1.26 for naltrexone related compound A, 1.80 for 2,2'-bisnaltrexone, and 1.99 for 10-ketonaltrexone; the resolution, R , between naltrexone and naltrexone related compound A is not less than 2.0; the tailing factor for the naltrexone peak is not greater than 1.4; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in

mg, of $C_{20}H_{23}NO_4 \cdot HCl$ in the portion of Naltrexone Hydrochloride taken by the formula:

$$(377.86/341.40)10C(r_U/r_S)$$

in which 377.86 and 341.40 are the molecular weights of naltrexone hydrochloride and naltrexone, respectively; C is the concentration, in mg per mL, of USP Naltrexone RS in the *Standard preparation*; and r_U and r_S are the peak responses of naltrexone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Naltrexone Hydrochloride Tablets

» Naltrexone Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of naltrexone hydrochloride ($C_{20}H_{23}NO_4 \cdot HCl$).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Naltrexone RS

USP Naltrexone Related Compound A RS

N-(3-Butenyl)-noroxymorphone hydrochloride.

$C_{20}H_{23}NO_4 \cdot HCl$ 377.87

Identification—The retention time of the major peak for naltrexone in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 60 minutes.

Determine the amount of $C_{20}H_{23}NO_4 \cdot HCl$ dissolved using the method described below.

0.05 M Buffer solution—Dissolve 7.0 g of monobasic sodium phosphate in 1 L of water.

Mobile phase—Prepare a mixture of 600 mL of 0.05 M *Buffer solution*, 1.1 g of sodium 1-octane sulfonate monohydrate and 400 mL of methanol. Adjust with dilute sodium hydroxide to a pH of 6.7 \pm 0.05, if necessary, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm \times 15-cm column that contains packing L1 and is heated to 45°. The flow rate is about 1 mL per minute. Is chromatograph replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

Procedure—Inject a volume (about 100 μ L) of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Calculate the amount of $C_{20}H_{23}NO_4 \cdot HCl$ dissolved in comparison with a *Standard solution* having a known concentration of USP Naltrexone RS in the same *Medium* and similarly chromatographed.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{20}H_{23}NO_4 \cdot HCl$ is dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Solution A, **Solution B**, **Mobile phase**, **Resolution solution**, **Standard preparation**, and **Chromatographic system**—Proceed as directed in the *Assay* under *Naltrexone Hydrochloride*.

Assay preparation—Transfer not fewer than 20 Tablets to a tared container, and determine the average Tablet weight.

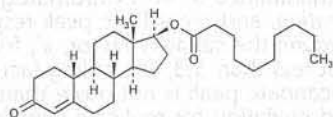
Grind the Tablets to a homogeneous mixture. Transfer an accurately weighed portion, equivalent to about 250 mg of naltrexone hydrochloride, to a 100-mL volumetric flask. Add about 80 mL of 0.1 M phosphoric acid, and shake or sonicate for at least 30 minutes. Dilute with 0.1 M phosphoric acid to volume, mix, and filter.

Procedure—Proceed as directed for *Procedure* in the Assay under *Naltrexone Hydrochloride*. Calculate the quantity, in mg, of naltrexone hydrochloride ($C_{20}H_{23}NO_4 \cdot HCl$) in the portion of Tablets taken by the formula:

$$(377.86/341.40)100C(r_U / r_S)$$

in which the terms are defined therein.

Nandrolone Decanoate



$C_{28}H_{44}O_3$ 428.65
Estr-4-en-3-one, 17-[(1-oxodecyl)oxy]-, (17 β)-.
17 β -Hydroxyestr-4-en-3-one decanoate [360-70-3].

» Nandrolone Decanoate contains not less than 97.0 percent and not more than 103.0 percent of $C_{28}H_{44}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store in a refrigerator.

USP Reference standards (11)—

USP Nandrolone RS
USP Nandrolone Decanoate RS

Completeness and clarity of solution—A solution in dioxane (1 in 50) is clear.

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 10 μ g per mL.

Medium: alcohol.

Absorptivities at 239 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: Prepare a solution in acetone containing 5 mg per mL. Apply 10 μ L of this solution and 10 μ L of a solution of USP Nandrolone Decanoate RS in acetone containing 5 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-heptane and acetone (3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with a solution of sulfuric acid in alcohol (1 in 50) and heating in an oven at 110° for 15 minutes: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Melting range (741): between 33° and 37°.

Specific rotation (781S): between +32° and +36°.

Test solution: 10 mg per mL, previously dried in dioxane.

Loss on drying (731)—Dry it in vacuum over silica gel for 4 hours: it loses not more than 0.5% of its weight.

Chromatographic purity—

Mobile phase—Prepare a filtered and degassed mixture of chromatographic *n*-heptane and *n*-propyl alcohol (HPLC grade) (97:3). Make adjustments if necessary (see *System suitability* under *Chromatography* (621)).

System suitability solution—Dissolve accurately weighed quantities of USP Nandrolone Decanoate RS, dimethyl phthalate, and USP Nandrolone RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 0.25 mg per mL, 0.25 mg per mL, and 0.16 mg per mL, respectively.

Test solution—Transfer about 13 mg of Nandrolone Decanoate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 238-nm detector and a 4.6-mm \times 25-cm column that contains packing L10. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.67 for dimethyl phthalate and 1.0 for nandrolone decanoate; the resolution, R , between dimethyl phthalate and nandrolone decanoate is not less than 9.0, and the nandrolone peak elutes before 4.5 times the elution time of nandrolone decanoate; the tailing factor is not more than 1.3 for the nandrolone decanoate and dimethyl phthalate peaks; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject a volume (about 20 μ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Nandrolone Decanoate taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response for each impurity, and r_s is the sum of the responses of all of the peaks: the sum of all impurities is not more than 3.0%.

Assay—[NOTE—Use low-actinic glassware throughout this procedure.]

Mobile phase—Prepare a filtered and degassed mixture of methanol and water (95:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Transfer an accurately weighed quantity of USP Nandrolone Decanoate RS to a suitable volumetric flask, and dilute with methanol to volume to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer an accurately weighed quantity of about 20 mg of Nandrolone Decanoate to a 100-mL volumetric flask. Dissolve in and dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and an 8-mm \times 10-cm analytical column containing packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor, k' , is not less than 1.3; the column efficiency is not less than 8000 theoretical plates; the tailing factor is not less than 0.9 and not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of $C_{28}H_{44}O_3$ in the portion of Nandrolone Decanoate taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Nandrolone Decanoate RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nandrolone Decanoate Injection

» Nandrolone Decanoate Injection is a sterile solution of Nandrolone Decanoate in Sesame Oil, with a suitable preservative. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nandrolone decanoate ($C_{28}H_{44}O_3$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards (11)—

USP Nandrolone RS

USP Nandrolone Decanoate RS

Identification—Dilute a volume of Injection with acetone to provide a solution containing approximately 5 mg of nandrolone decanoate per mL. This solution responds to *Identification test C* under *Nandrolone Decanoate*, 5- μ L portions of the test solution and the Standard solution being used.

Limit of nandrolone—

Standard preparation—Dissolve 25.0 mg of USP Nandrolone RS in 50.0 mL of acetone. Dilute 5.0 mL of this solution with acetone to 50.0 mL, and mix.

Test preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of nandrolone decanoate, to a 10-mL volumetric flask, dilute with acetone to volume, and mix.

Procedure—Apply 10 μ L each of the *Standard preparation* and of the *Test preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-heptane and acetone (3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Return the dry plate to the developing chamber containing the same solvent system, and again develop the chromatogram until the solvent front has moved the same distance from the origin. Remove the plate from the developing chamber, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with a 4 in 10 solution of sulfuric acid in methanol and heating at about 100° for 10 minutes. Cool, and examine under long-wavelength UV light: any yellow fluorescent spot from the *Test preparation* at an R_f value of about 0.2 is not greater in size or intensity than that produced by the *Standard preparation* at the same R_f value, corresponding to not more than 1.0% of nandrolone.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

0.02 M Ammonium acetate solution—Transfer about 1.6 g of ammonium acetate to a 1-liter volumetric flask. Dissolve in and dilute with water to volume.

Mobile phase—Prepare a filtered and degassed mixture of alcohol and 0.02 M Ammonium acetate solution (66:34). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Nandrolone Decanoate RS with tetrahydrofuran, and dilute quantitatively and stepwise if necessary, with tetrahydrofuran to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 400 mg of nandrolone decanoate to a 200-mL volumetric flask, dilute with tetrahydrofuran to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with tetrahydrofuran to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm \times 15-cm column containing 5- μ m packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 40°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor, k' , for nandrolone decanoate is not less than 5.3; the tailing factor for the Nandrolone Decanoate peak is not more than 1.4; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{28}H_{44}O_3$ in each mL of the Injection taken by the formula:

$$2000(C/V) (r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Nandrolone Decanoate RS in the *Standard preparation*; V is the volume, in mL, of the injection taken to prepare the *Assay preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nandrolone Phenpropionate

$C_{27}H_{34}O_3$ 406.56

Estr-4-en-3-one, 17-(1-oxo-3-phenylpropoxy)-, (17 β)-

17 β -Hydroxyestr-4-en-3-one hydrocinnamate [62-90-8].

» Nandrolone Phenpropionate contains not less than 97.0 percent and not more than 103.0 percent of $C_{27}H_{34}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Nandrolone Phenpropionate RS

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 10 μ g per mL.

Medium: alcohol.

Absorptivities at 239 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: Prepare a solution in acetone containing 5 mg per mL. Apply 10 μ L of this solution and 10 μ L of a solution of USP Nandrolone Phenpropionate RS in acetone containing 5 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and de-

velop the chromatogram in a solvent system consisting of a mixture of *n*-heptane and acetone (2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with a 1 in 50 mixture of sulfuric acid in alcohol and heating at 110° for 15 minutes: the R_f value of the principal spot obtained from the test solution corresponds to that obtained from the *Standard solution*.

Melting range (741): between 95° and 99°.

Specific rotation (781S): between +48° and +51°.

Test solution: 20 mg per mL, in dioxane.

Loss on drying (731)—Dry it in a suitable vacuum drying tube, using phosphorus pentoxide as the desiccant, at 80° for 3 hours: it loses not more than 0.5% of its weight.

Assay—

Standard preparation—Prepare as directed under *Single-Steroid Assay* (511), using USP Nandrolone Phenpropionate RS.

Assay preparation—Weigh accurately about 20 mg of Nandrolone Phenpropionate, previously dried, dissolve it in a sufficient quantity of a mixture of equal volumes of alcohol and chloroform to make 10.0 mL, and mix.

Procedure—Proceed as directed for *Procedure under Single-Steroid Assay* (511), using a solvent system consisting of a mixture of *n*-heptane and acetone (3:1), through the fourth sentence of the second paragraph under *Procedure*. Then centrifuge the tubes for 5 minutes, and determine the absorbances of the supernatants in 1-cm cells at the wavelength of maximum absorbance at about 239 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_{27}H_{34}O_3$ in the portion of Nandrolone Phenpropionate taken by the formula:

$$10C(A_U / A_S)$$

in which *C* is the concentration, in mg per mL, of USP Nandrolone Phenpropionate RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Nandrolone Phenpropionate Injection

» Nandrolone Phenpropionate Injection is a sterile solution of Nandrolone Phenpropionate in a suitable oil. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nandrolone phenpropionate ($C_{27}H_{34}O_3$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards (11)—

USP Nandrolone RS

Identification—Dilute the Injection with acetone to obtain a solution containing 5 mg of nandrolone phenpropionate in each mL. Proceed as directed for *Identification test C* under *Nandrolone Phenpropionate*, beginning with "Apply 10 μ L of this solution."

Limit of nandrolone—

Standard preparation—Prepare as directed in the test for *Limit of nandrolone* under *Nandrolone Decanoate Injection*.

Test preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of nandrolone phenpropionate, to a 10-mL volumetric flask, dilute with acetone to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the test for *Limit of nandrolone* under *Nandrolone Decanoate Injection*.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Isoniazid reagent—Dissolve 500 mg of isoniazid in about 250 mL of methanol, add 0.63 mL of hydrochloric acid, dilute with methanol to 500.0 mL, and mix.

Standard preparation—Transfer about 25 mg of USP Nandrolone Phenpropionate RS, accurately weighed, to a 100-mL volumetric flask, dissolve in chloroform, dilute with chloroform to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with chloroform to volume, and mix.

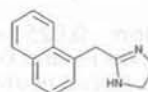
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of nandrolone phenpropionate, to a 200-mL volumetric flask, dilute with chloroform to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with chloroform to volume, and mix.

Procedure—Transfer 5.0 mL each of the *Standard preparation*, of the *Assay preparation*, and of chloroform to provide the blank, to separate 10-mL volumetric flasks, dilute each flask with *Isoniazid reagent* to volume, and mix. Allow the flasks to stand for 1 hour with occasional shaking. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 380 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_{27}H_{34}O_3$ in each mL of the Injection taken by the formula:

$$(2C / V)(A_U / A_S)$$

in which *C* is the concentration, in μ g per mL, of USP Nandrolone Phenpropionate RS in the *Standard preparation*, *V* is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Naphazoline Hydrochloride



$C_{14}H_{14}N_2 \cdot HCl$ 246.74
1*H*-Imidazole, 4,5-dihydro-2-(1-naphthalenylmethyl)-, monohydrochloride;
2-(1-Naphthylmethyl)-2-imidazoline monohydrochloride [550-99-2].

DEFINITION

Naphazoline Hydrochloride contains NLT 98.0% and NMT 102.0% of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (17K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191)

Sample solution: 10 mg/mL in water

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Buffer: In a 1000-mL volumetric flask, dissolve 3.0 g of monobasic potassium phosphate in 800 mL of water.

Add 3.0 mL of triethylamine, adjust with phosphoric acid to a pH of 3.0, and dilute with water to volume.

Mobile phase: Acetonitrile and Buffer (20:80)

Standard solution: 0.05 mg/mL of USP Naphazoline Hydrochloride RS in water

Sample solution: 0.05 mg/mL of Naphazoline Hydrochloride in water

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) in the portion of Naphazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Naphazoline Hydrochloride RS in the Standard solution (mg/mL)

C_U = concentration of Naphazoline Hydrochloride in the Sample solution (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.2%

• **ORGANIC IMPURITIES**

Solution A: Acetonitrile, glacial acetic acid, and water (30:0.5:70)

Mobile phase: 1.1 g/L of anhydrous sodium 1-octanesulfonate in Solution A

System suitability solution: 0.025 mg/mL of USP Naphazoline Hydrochloride RS and 0.05 mg/mL of 1-naphthylacetic acid in Mobile phase

Standard solution: 0.5 µg/mL each of USP Naphazoline Hydrochloride RS and USP Naphazoline Related Compound A RS in Mobile phase

Sample solution: 0.5 mg/mL of Naphazoline Hydrochloride in Mobile phase

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm × 25-cm; 3-µm or 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 3 times the retention time of the naphazoline peak

System suitability

Sample: System suitability solution

Suitability requirements

Resolution: NLT 5.0 between the naphazoline and 1-naphthylacetic acid peaks

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of naphazoline related compound A in the portion of Naphazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naphazoline related compound A from the Sample solution

r_S = peak response of naphazoline related compound A from the Standard solution

C_S = concentration of USP Naphazoline Related Compound A RS in the Standard solution (mg/mL)

C_U = concentration of Naphazoline Hydrochloride in the Sample solution (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Naphazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the Sample solution

r_S = peak response of naphazoline from the Standard solution

C_S = concentration of USP Naphazoline Hydrochloride RS in the Standard solution (mg/mL)

C_U = concentration of Naphazoline Hydrochloride in the Sample solution (mg/mL)

Acceptance criteria

Individual impurities: See Table 1. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Naphazoline related compound A	0.76	0.1
Naphazoline	1.0	—
1-Naphthylacetic acid	1.4	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS

• **pH** (791)

Sample solution: 10 mg/mL in carbon dioxide-free water

Acceptance criteria: 5.0–6.6. The Sample solution is clear and colorless.

• **LOSS ON DRYING** (731)

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** (11)

USP Naphazoline Hydrochloride RS

USP Naphazoline Related Compound A RS

N-(2-Aminoethyl)-2-(naphthalen-1-yl)acetamide.

$C_{14}H_{16}N_2O$ 228.29

Naphazoline Hydrochloride Nasal Solution

DEFINITION

Naphazoline Hydrochloride Nasal Solution is a solution of Naphazoline Hydrochloride in water adjusted to a suitable pH and tonicity. It contains NLT 90.0% and NMT 110.0% of the labeled amount of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$).

IDENTIFICATION

- A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 400 mL of water. Add 250 mL of acetonitrile and 10 mL of glacial acetic acid, and dilute with water to 1000 mL. Sonicate for 10 min to obtain a solution having a pH of about 3.5.

Standard solution: 0.25 mg/mL of USP Naphazoline Hydrochloride RS in water

Sample solution: Equivalent to 0.25 mg/mL of naphazoline hydrochloride in water from Nasal Solution

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4-mm \times 30-cm; packing L11

Flow rate: 2 mL/min

Injection volume: 15 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Naphazoline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of naphazoline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- USP REFERENCE STANDARDS (11)**
USP Naphazoline Hydrochloride RS

Naphazoline Hydrochloride Ophthalmic Solution

DEFINITION

Naphazoline Hydrochloride Ophthalmic Solution is a sterile, buffered solution of Naphazoline Hydrochloride in water adjusted to a suitable tonicity. It contains NLT 90.0% and NMT 115.0% of the labeled amount of naphazoline hy-

drochloride ($C_{14}H_{14}N_2 \cdot HCl$). It contains a suitable preservative.

IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Buffer: Dissolve 3 g of monobasic potassium phosphate in 1 L of water, and add 3 mL of triethylamine. Adjust with phosphoric acid to a pH of 3.

Mobile phase: Acetonitrile and *Buffer* (20:80)

Standard solution: 0.05 mg/mL of USP Naphazoline Hydrochloride RS in *Mobile phase*

Sample solution: Equivalent to 0.05 mg/mL of naphazoline hydrochloride in *Mobile phase* from Ophthalmic Solution

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 285 nm or diode array. [NOTE—Use diode array detector to perform *Identification test B*.]

Column: 4.6-mm \times 15-cm; packing L10

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Naphazoline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of naphazoline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

- pH (791):** 5.5–7.0
- STERILITY TESTS (71):** Meets the requirements

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers.
- USP REFERENCE STANDARDS (11)**
USP Naphazoline Hydrochloride RS

Naphazoline Hydrochloride and Pheniramine Maleate Ophthalmic Solution

DEFINITION

Naphazoline Hydrochloride and Pheniramine Maleate Ophthalmic Solution is a sterile, buffered solution of Naphazoline Hydrochloride and Pheniramine Maleate in water adjusted to a suitable tonicity. It contains NLT

90.0% and NMT 110.0% of the labeled amount of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) and pheniramine maleate ($C_{16}H_{20}N_2 \cdot C_4H_4O_4$). It contains a suitable preservative.

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHY

Standard solution A: 1.5 mg/mL of USP Naphazoline Hydrochloride RS in water

Standard solution B: 6.0 mg/mL of USP Pheniramine Maleate RS in water

Sample solution: Equivalent to 0.25 mg/mL of naphazoline hydrochloride and 3 mg/mL of pheniramine maleate in water from Ophthalmic Solution

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Adsorbent: 0.25-mm layer of silica gel on a 20-cm \times 20-cm chromatographic plate

Application volume: 5 μ L of *Standard solution A*, 10 μ L of *Standard solution B*, and 30 μ L of the *Sample solution*

Developing solvent system: Methanol, acetic acid, and water (8:1:1)

Spray reagent: Ninhydrin TS

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Allow the spots to dry, then place the plate in a saturated chromatographic chamber, and develop in the *Developing solvent system* until the solvent front has moved to 1.5 cm from the top of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Spray with *Spray reagent*, and place in an oven at 105° to visualize the spots. Both the naphazoline and pheniramine spots are purplish gray in color.

Acceptance criteria: The R_f values of the spots of the *Sample solution* correspond to those of *Standard solution A* and *Standard solution B*.

- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer solution: Dissolve 14.2 g of anhydrous dibasic sodium phosphate and 20 mL of triethylamine in 1900 mL of water. Adjust with phosphoric acid to a pH of 5.6 ± 0.1 , and dilute with water to 2000 mL.

Mobile phase: Acetonitrile and *Buffer solution* (20:80)

Standard stock solution A: 0.75 mg/mL of USP Naphazoline Hydrochloride RS in *Mobile phase*

Standard stock solution B: 3.00 mg/mL of USP Pheniramine Maleate RS in *Mobile phase*

Standard solution: 0.03 mg/mL of naphazoline hydrochloride and 0.36 mg/mL of pheniramine maleate in *Mobile phase* prepared as follows. Transfer 1.0 mL of *Standard stock solution A* and 3.0 mL of *Standard stock solution B* to a 25-mL volumetric flask. Dilute with *Mobile phase* to volume.

Sample solution: Transfer a volume of Ophthalmic Solution, equivalent to 0.75 mg of naphazoline hydrochloride and 9.0 mg of pheniramine maleate, to a 25-mL volumetric flask. Dilute with *Mobile phase* to volume.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm \times 15-cm; packing L7

Flow rate: 1.5 mL/min

Injection volume: 25 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2 between the naphazoline and pheniramine peaks

Column efficiency: NLT 750 theoretical plates for the naphazoline and pheniramine peaks

Tailing factor: NMT 2.5 for pheniramine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naphazoline from the *Sample solution*

r_S = peak response of naphazoline from the *Standard solution*

C_S = concentration of USP Naphazoline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of naphazoline hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of pheniramine maleate ($C_{16}H_{20}N_2 \cdot C_4H_4O_4$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pheniramine from the *Sample solution*

r_S = peak response of pheniramine from the *Standard solution*

C_S = concentration of USP Pheniramine Maleate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pheniramine maleate in the *Sample solution* (mg/mL)

Acceptance criteria

Naphazoline hydrochloride: 90.0%–110.0%

Pheniramine maleate: 90.0%–110.0%

SPECIFIC TESTS

- **pH (791):** 5.7–6.3

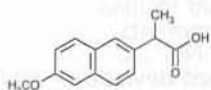
- **STERILITY TESTS (71):** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, Membrane Filtration.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature between 20° and 25°, protected from light.

- **USP REFERENCE STANDARDS (11)**
USP Naphazoline Hydrochloride RS
USP Pheniramine Maleate RS

Naproxen



$C_{14}H_{14}O_3$ 230.26
2-Naphthaleneacetic acid, 6-methoxy- α -methyl-, (S)-
(+)-(S)-6-Methoxy- α -methyl-2-naphthaleneacetic acid
[22204-53-1].

» Naproxen contains not less than 98.5 percent and not more than 101.5 percent of $C_{14}H_{14}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Naproxen RS

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 25 μ g per mL.

Medium: methanol.

Absorptivities at 271 nm, calculated on the dried basis, do not differ by more than 3%.

Specific rotation (781S): between +83.0° and +89.5°.

Test solution: 10 mg per mL, in methyl isobutyl ketone.

Loss on drying (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Delete the following:

• **Heavy metals, Method II (231):** 0.002%. (Official 1-Jan-2018)

Chromatographic purity—Dissolve 100 mg of Naproxen in methanol, and dilute with methanol to 5.0 mL to obtain the *Test solution*. Dissolve a suitable quantity of USP Naproxen RS in methanol to obtain a *Standard solution* having a known concentration of about 20 mg per mL. Dilute a portion of this solution quantitatively and stepwise with methanol to obtain three *Comparison solutions* having concentrations of 20, 60, and 100 μ g per mL (0.1%, 0.3%, and 0.5% of the *Standard solution*), respectively. Apply separate 10- μ L portions of the five solutions to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography (621)*) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of toluene, tetrahydrofuran, and glacial acetic acid (30:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, air-dry, and view under short-wavelength UV light: the R_f value of the principal spot in the chromatogram of the *Test solution* corresponds to that of the *Standard solution*, and any other spot obtained from the *Test solution* does not exceed, in size or intensity, the principal spot obtained from the 100- μ g-per-mL *Comparison solution* (0.5%), and the sum of the intensities of any secondary spots, similarly compared, does not exceed 2.0%.

Assay—Dissolve about 500 mg of Naproxen, accurately weighed, in a mixture of 75 mL of methanol and 25 mL of water that has been previously neutralized to the phenolphthalein endpoint with 0.1 N sodium hydroxide. Dissolve by gentle warming, if necessary, add phenolphthalein TS,

and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 23.03 mg of $C_{14}H_{14}O_3$.

Naproxen Oral Suspension

DEFINITION

Naproxen Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of naproxen ($C_{14}H_{14}O_3$).

IDENTIFICATION

• A.

Sample solution: *Standard solution* and *Sample solution* (1:1), prepared as directed in the *Assay*

Analysis: Chromatograph as directed in the *Assay*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits two main peaks corresponding to naproxen and the internal standard.

ASSAY

• PROCEDURE

Mobile phase: Prepare a mixture of 500 mL of methanol, 500 mL of water, and 2.46 g of anhydrous sodium acetate, and mix until dissolved. Adjust with glacial acetic acid to a pH of 5.8.

Internal standard solution: 1.1 mg/mL of ethylparaben in methanol

Standard stock solution: Transfer about 62.5 mg of USP Naproxen RS, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of methanol, and sonicate to dissolve. Add 5.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

Standard solution: 0.05 mg/mL of USP Naproxen RS and 0.0044 mg/mL of ethylparaben in *Mobile phase* from *Standard stock solution*

Sample stock solution: Transfer an accurately measured volume of Oral Suspension, previously well-mixed and free from air bubbles, nominally equivalent to about 125 mg of naproxen, to a 100-mL volumetric flask, using a "to contain" pipet. Rinse the pipet several times with methanol, and add the rinsings to the volumetric flask. Add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

Sample solution: Nominally equivalent to 0.05 mg/mL of naproxen and 0.0044 mg/mL of ethylparaben in *Mobile phase* from *Sample stock solution*. Filter if necessary to obtain a clear solution.

Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 35 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ethylparaben and naproxen are 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between ethylparaben and naproxen

Tailing factor: NMT 2.0 for the naproxen peak

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naproxen ($C_{14}H_{14}O_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of naproxen to ethylparaben from the *Sample solution*

- R_s = peak response ratio of naproxen to ethylparaben from the *Standard solution*
 C_s = concentration of USP Naproxen RS in the *Standard solution* (mg/mL)
 C_u = nominal concentration of naproxen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DELIVERABLE VOLUME** (698): Meets the requirements for oral suspension packaged in multiple-unit containers
- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements for oral suspension packaged in single-unit containers

SPECIFIC TESTS

- **PH** (791): 2.2–3.7

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
- **USP REFERENCE STANDARDS** (11)
USP Naproxen RS

Naproxen Tablets

DEFINITION

Naproxen Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of naproxen ($C_{14}H_{14}O_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B.** The UV absorption spectra of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the Assay.

ASSAY

PROCEDURE

Mobile phase: Acetonitrile, water, and glacial acetic acid (450:540:10)

Standard solution: 0.1 mg/mL of USP Naproxen RS in *Mobile phase*

Sample stock solution: Nominally equivalent to 1 mg/mL of naproxen in *Mobile phase*. Transfer an amount equivalent to about 500 mg of naproxen, from NLT 10 finely powdered Tablets, to a 500-mL volumetric flask. Add about 300 mL of *Mobile phase*, and sonicate for 30 min. Cool to room temperature, and dilute with *Mobile phase* to volume.

Sample solution: Nominally equivalent to 0.1 mg/mL of naproxen in *Mobile phase* from *Sample stock solution*. Pass through a suitable filter of 0.45- μ m of pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254-nm diode array

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Flow rate: 1.2 mL/min

Injection volume: 20 μ L

Run time: NLT 2 times the retention time of naproxen

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NLT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naproxen ($C_{14}H_{14}O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of naproxen from the *Sample solution*

r_s = peak response of naproxen from the *Standard solution*

C_s = concentration of USP Naproxen RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of naproxen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Buffer: 0.1 M of pH 7.4 phosphate buffer prepared as follows. Dissolve 2.62 g of monobasic sodium phosphate and 11.50 g of anhydrous dibasic sodium phosphate in 1000 mL of water, and mix.

Medium: *Buffer*, 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: A known concentration of USP Naproxen RS in *Buffer*

Sample solution: Filter portions of the solution under test, and suitably dilute with *Buffer*.

Instrumental conditions

Mode: UV

Analytical wavelength: About 332 nm (maximum absorbance)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naproxen ($C_{14}H_{14}O_3$) dissolved.

Tolerances: NLT 80% (Q) of the labeled amount of naproxen ($C_{14}H_{14}O_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

IMPURITIES

ORGANIC IMPURITIES

Buffer: Dissolve 1.36 g of monobasic potassium phosphate in 1 L of water. Adjust with triethylamine to a pH of 6.5. Pass through a suitable filter of 0.45- μ m pore size.

Diluent: Acetonitrile and *Buffer* (50:50)

Mobile phase: See Table 1.

Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	85	15
5	85	15
25	60	40
45	50	50

Table 1 (Continued)

Time (min)	Buffer (%)	Acetonitrile (%)
50	85	15
60	85	15

Standard stock solution 1: Prepare 5 mg/mL of USP Naproxen RS in *Diluent*. Further dilute this solution with *Diluent* to obtain 0.05 mg/mL of USP Naproxen RS in *Diluent*.

Standard stock solution 2: 0.01 mg/mL of USP Naproxen Related Compound A RS in methanol

Standard stock solution 3: 0.01 mg/mL of USP Naproxen Related Compound L RS in methanol

System suitability solution: 0.5 mg/mL of USP Naproxen RS and 0.5 µg/mL of USP Naproxen Related Compound A RS in *Diluent*, from *Standard stock solution 1* and *Standard stock solution 2*, respectively

Standard solution: 1.0 µg/mL of USP Naproxen RS, 0.5 µg/mL each of USP Naproxen Related Compound A RS and USP Naproxen Related Compound L RS in *Diluent*, from *Standard stock solution 1*, *Standard stock solution 2*, and *Standard stock solution 3*, respectively

Sample solution: Nominally equivalent to 0.5 mg/mL of naproxen from NLT 10 finely powdered Tablets. Transfer nominally equivalent to about 500 mg of naproxen to a 1000-mL volumetric flask. Add 600 mL of *Diluent*, and sonicate for 30 min with intermittent shaking. Cool to room temperature, and dilute with *Diluent* to volume. Mix, and allow to settle for 5 min. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 236 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 6.0 between naproxen related compound A and naproxen, *System suitability solution*

Relative standard deviation: NMT 5.0% for naproxen, naproxen related compound A, and naproxen related compound L, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of naproxen related compound A and naproxen related compound L in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_U/C_S) \times 100$$

r_U = peak response of naproxen related compound A or naproxen related compound L from the *Sample solution*

r_S = peak response of naproxen related compound A or naproxen related compound L from the *Standard solution*

C_S = concentration of USP Naproxen Related Compound A RS or USP Naproxen Related Compound L RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of naproxen in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of any other individual impurity from the *Sample solution*

r_S = peak response of naproxen from the *Standard solution*

C_S = concentration of USP Naproxen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of naproxen in the *Sample solution* (mg/mL)

F = relative response factor of each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Naproxen related compound A ^a	0.63	—	0.10
Naproxen	1.00	—	—
Naproxen related compound L ^b	2.32	—	0.10
Naproxen methyl ester ^c	3.19	1.0	0.10
Any other individual impurity	—	1.0	0.10
Total impurities ^d	—	—	0.50

^a 6-Methoxy-2-naphthoic acid.

^b 1-(6-Methoxynaphthalen-2-yl)ethanone.

^c (S)-Methyl 2-(6-methoxynaphthalen-2-yl)propanoate.

^d Disregard any peaks below LOQ (0.004% for any other individual impurity and naproxen methyl ester, 0.002% for naproxen related compound A, and 0.006% for naproxen related compound L).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**

USP Naproxen RS

USP Naproxen Related Compound A RS

6-Methoxy-2-naphthoic acid.

C₁₂H₁₀O₃ 202.21

USP Naproxen Related Compound L RS

1-(6-Methoxynaphthalen-2-yl)ethanone.

C₁₃H₁₂O₂ 200.23

Naproxen Delayed-Release Tablets

DEFINITION

Naproxen Delayed-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of naproxen (C₁₄H₁₄O₃).

IDENTIFICATION

• **A. ULTRAVIOLET ABSORPTION** <197U>

Standard solution and Sample solution: Prepare as directed in the *Buffer stage* of the *Dissolution* test.

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile and 1% acetic acid solution (900:1100). Filter and degas.

Diluent A: Acetonitrile and water (9:1)

Diluent B: Acetonitrile and water (1:1)

Standard stock solution: 0.5 mg/mL of USP Naproxen RS in *Diluent A*

Standard solution: 0.1 mg/mL of USP Naproxen RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Transfer an amount nominally equivalent to 250 mg of naproxen from 20 powdered Tablets into a 100-mL volumetric flask, and add about 70 mL of *Diluent B*. Shake by mechanical means for 15 min, sonicate for 15 min, dilute with *Diluent B* to volume, and mix. Pass this solution through a suitable filter of 0.45- μ m pore size. Transfer 2.0 mL of the filtrate into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1.0 mL/min

Injection volume: 50 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of naproxen ($C_{14}H_{14}O_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of naproxen from the *Sample solution*

r_S = peak response of naproxen from the *Standard solution*

C_S = concentration of USP Naproxen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of naproxen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION, Delayed-Release Dosage Forms, Method B (711)

Acid stage

Medium: 0.1 N hydrochloric acid; 1000 mL

Apparatus 2: 50 rpm

Time: 2 h

Standard solution: A known concentration of USP Naproxen RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* if necessary.

Analysis: Determine the amount of naproxen ($C_{14}H_{14}O_3$) dissolved by UV absorption at the wavelength of maximum absorbance at about 332 nm with the *Sample solution* in comparison with the *Standard solution*.

Tolerances: NMT 10% (Q) of the labeled amount of naproxen ($C_{14}H_{14}O_3$) is dissolved.

Buffer stage

Buffer: 0.2 M phosphate buffer, pH 6.8

Medium: *Buffer*; 1000 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: A known concentration of USP Naproxen RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium* if necessary.

Analysis: Determine the amount of naproxen ($C_{14}H_{14}O_3$) dissolved by UV absorption at the wavelength of maximum absorbance at about 332 nm with the *Sample solution* in comparison with the *Standard solution*.

Tolerances: NLT 80% (Q) of the labeled amount of naproxen ($C_{14}H_{14}O_3$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Mobile phase, Diluent A, Diluent B, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard stock solution: 0.25 mg/mL of USP Naproxen RS in *Diluent A*

Standard solution: 0.1 mg/mL of USP Naproxen RS in *Diluent B* from *Standard stock solution*

Sample solution: Transfer 1 Tablet to a 200-mL volumetric flask, and add 140 mL of *Diluent B*. Shake by mechanical means for 15 min, sonicate for 15 min, and dilute with *Diluent B* to volume. Pass a portion of this solution through a suitable filter of 0.45- μ m pore size, pipet 2.0 mL of the filtrate for a 500-mg tablet and 2.5 mL for a 375-mg tablet into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**
USP Naproxen RS

Naproxen Sodium

$C_{14}H_{13}NaO_3$ 252.24

2-Naphthaleneacetic acid, 6-methoxy- α -methyl-, sodium salt, (S)-.

(-)-Sodium (S)-6-methoxy- α -methyl-2-naphthaleneacetate [26159-34-2].

» Naproxen Sodium contains not less than 98.0 percent and not more than 102.0 percent of $C_{14}H_{13}NaO_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Naproxen Sodium RS

Identification—

A: *Infrared Absorption* (197K).

B: *Ultraviolet Absorption* (197U)—

Solution: 25 μ g per mL.

Medium: methanol.

Absorptivities at 272 nm, calculated on the dried basis, do not differ by more than 3%.

Specific rotation (781S): between -15.3° and -17.0° .

Test solution: 50 mg per mL, in 0.1 N sodium hydroxide.

Loss on drying (731)—Dry it in vacuum at 105° for 3 hours: it loses not more than 1.0% of its weight.

Delete the following:

• **Heavy metals, Method I** (231)—Dissolve 1.0 g in 20 mL of water in a separator, add 5 mL of 1 N hydrochloric acid, and extract with successive 20-mL, 20-mL, and 10-mL portions of methylene chloride. Discard the methylene chloride extracts, and use the aqueous layer for the test: the limit is 0.002%. • (Official 1-Jan-2018)

Free naproxen—Dissolve about 5.0 g in 25 mL of water in a separator, and extract the solution with three 15-mL portions of chloroform. Evaporate the combined extracts on a steam bath to dryness. Dissolve the residue in 10 mL of a mixture of methanol and water (3:1) previously neutralized with 0.1 N sodium hydroxide to the phenolphthalein

endpoint. Add phenolphthalein TS, and titrate with 0.10 N sodium hydroxide: not more than 2.2 mL is consumed (1.0%).

Chromatographic purity—Dissolve 100 mg in 5 mL of methanol. Dissolve a suitable quantity of USP Naproxen Sodium RS in methanol to obtain a *Standard solution* having a known concentration of about 20 mg per mL. Dilute a portion of this solution quantitatively with methanol to obtain three *Comparison solutions* having concentrations of 20, 60, and 100 µg per mL (0.1%, 0.3%, and 0.5% of the *Standard solution*), respectively. Apply separate 10-µL portions of the five solutions on the starting line to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of toluene, tetrahydrofuran, and glacial acetic acid (30:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, air-dry, and view under short-wavelength UV light: the R_f value of the principal spot in the chromatogram of the solution under test corresponds to that of the *Standard solution*, the intensity of any individual secondary spot does not exceed that of the 100-µg-per-mL *Comparison solution* (0.5%), and the sum of the intensities of any secondary spots, similarly compared, does not exceed 2.0%.

Assay—Dissolve about 200 mg of Naproxen Sodium, accurately weighed, in 50 mL of glacial acetic acid containing 2 drops of *p*-naphtholbenzein TS previously neutralized with 0.1 N perchloric acid if necessary. Titrate with 0.1 N perchloric acid VS. Each mL of 0.1 N perchloric acid is equivalent to 25.22 mg of $C_{14}H_{13}NaO_3$.

Naproxen Sodium Tablets

DEFINITION

Naproxen Sodium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$).

IDENTIFICATION

• A. IDENTIFICATION TESTS—GENERAL, Sodium (191)

Sample: Transfer an amount nominally equivalent to about 250 mg of naproxen sodium from finely powdered Tablets to a centrifuge tube. Add 12 mL of water and 1 mL of hydrochloric acid. A dense white precipitate is formed. Centrifuge the mixture. Use the clear supernatant for the test.

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **C.** The UV absorption spectra of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the Assay.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile, water, and glacial acetic acid (450:540:10)

Standard solution: 0.1 mg/mL of USP Naproxen Sodium RS in *Mobile phase*

Sample stock solution: Nominally equivalent to 5.5 mg/mL of naproxen sodium. Transfer an amount nominally equivalent to about 2750 mg of naproxen sodium, from NLT 5 Tablets, to a 500-mL volumetric flask. Add 75 mL of water, and sonicate till the Tablets disperse. Add about 250 mL of *Mobile phase*, and sonicate for 30 min with intermittent shaking. Cool to room

temperature, and dilute with *Mobile phase* to volume. Pass through a suitable filter of 0.45-µm pore size.

Sample solution: Nominally equivalent to 0.1 mg/mL of naproxen sodium in *Mobile phase* from *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254-nm diode array

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.2 mL/min

Injection volume: 20 µL

Run time: NLT 2 times the retention time of naproxen

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of naproxen from the *Sample solution*

r_s = peak response of naproxen from the *Standard solution*

C_s = concentration of USP Naproxen Sodium RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of naproxen sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Buffer: 0.1 M of pH 7.4 phosphate buffer containing 2.62 g/L of monobasic sodium phosphate and 11.50 g/L of anhydrous dibasic sodium phosphate in water

Medium: *Buffer*; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: 50 µg/mL of USP Naproxen Sodium RS in *Medium*

Sample solution: Dilute a filtered portion of the solution under test with *Medium* as necessary to obtain a nominal concentration of 50 µg/mL naproxen sodium ($C_{14}H_{13}NaO_3$).

Instrumental conditions

Mode: UV

Analytical wavelength: About 332 nm (maximum absorbance)

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$) dissolved.

Tolerances: NLT 80% (Q) of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Buffer: Dissolve 1.36 g of monobasic potassium phosphate in 1 L of water. Adjust with triethylamine to a pH of 6.5. Pass through a suitable filter of 0.45-µm pore size.

Diluent: Acetonitrile and *Buffer* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	85	15
5	85	15
25	60	40
45	50	50
50	85	15
60	85	15

Standard stock solution 1: Prepare 5 mg/mL of USP Naproxen Sodium RS in *Diluent*. Further dilute this solution with *Diluent* to obtain 0.05 mg/mL of USP Naproxen Sodium RS in *Diluent*.

Standard stock solution 2: 0.01 mg/mL of USP Naproxen Related Compound A RS in methanol

Standard stock solution 3: 0.01 mg/mL of USP Naproxen Related Compound L RS in methanol

Standard solution: 1.0 µg/mL of USP Naproxen Sodium RS, and 0.5 µg/mL each of USP Naproxen Related Compound A RS and USP Naproxen Related Compound L RS in *Diluent*, from *Standard stock solution 1*, *Standard stock solution 2*, and *Standard stock solution 3*, respectively

System suitability solution: 0.5 mg/mL of USP Naproxen Sodium RS and 0.5 µg/mL of USP Naproxen Related Compound A RS in *Diluent*, from *Standard stock solution 1* and *Standard stock solution 2*, respectively

Sample stock solution: Nominally equivalent to 5.5 mg/mL of naproxen sodium from NLT 5 finely powdered Tablets. Transfer nominally equivalent to about 2750 mg of naproxen sodium to a 500-mL volumetric flask. Add 250 mL of *Diluent*, and sonicate for 20 min with intermittent shaking. Cool to room temperature and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size.

Sample solution: Nominally equivalent to 0.55 mg/mL of naproxen sodium in *Diluent* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 236 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 6.0 between naproxen related compound A and naproxen, *System suitability solution*

Relative standard deviation: NMT 5.0% for naproxen, naproxen related compound A, and naproxen related compound L; *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of naproxen related compound A and naproxen related compound L in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_u/C_s) \times 100$$

r_u = peak response of naproxen related compound A or naproxen related compound L from the *Sample solution*

r_s = peak response of naproxen related compound A or naproxen related compound L from the *Standard solution*

C_s = concentration of USP Naproxen Related Compound A RS or USP Naproxen Related Compound L RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of naproxen sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of any other individual impurity from the *Sample solution*

r_s = peak response of naproxen from the *Standard solution*

C_s = concentration of USP Naproxen Sodium RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of naproxen sodium in the *Sample solution* (mg/mL)

F = relative response factor of individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Naproxen related compound A ^a	0.63	—	0.2
Naproxen	1.00	—	—
Naproxen related compound L ^b	2.32	—	0.2
Naproxen methyl ester ^c	3.19	1.0	0.2
Any other individual impurity	—	1.0	0.2
Total impurities ^d	—	—	1.5

^a 6-Methoxy-2-naphthoic acid.

^b 1-(6-Methoxynaphthalen-2-yl)ethanone.

^c (S)-Methyl 2-(6-methoxynaphthalen-2-yl)propanoate.

^d Disregard any peaks below LOQ (0.004% for any other individual impurity and naproxen methyl ester, 0.002% for naproxen related compound A, and 0.006% for naproxen related compound L).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**

USP Naproxen Sodium RS

USP Naproxen Related Compound A RS

6-Methoxy-2-naphthoic acid.

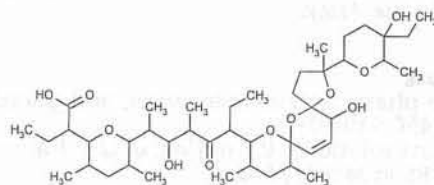
C₁₂H₁₀O₃ 202.21

USP Naproxen Related Compound L RS

1-(6-Methoxynaphthalen-2-yl)ethanone.

C₁₃H₁₂O₂ 200.23

Narasin Granular



C₄₃H₇₂O₁₁ (narasin A) 765.03

C₄₃H₇₀O₁₁ (narasin B) 763.01

C₄₄H₇₄O₁₁ (narasin D) 779.05

C₄₄H₇₄O₁₁ (narasin I) 779.05

Narasin.

2H-Pyran-2-acetic acid, α -ethyl-6-[5-[2-(5-ethyltetrahydro-5-hydroxy-6-methyl-2H-pyran-2-yl)-15-hydroxy-2,10,12-trimethyl-1,6,8-trioxadispiro[4.1.5.3]pentadec-13-en-9-yl]-2-hydroxy-1,3-dimethyl-4-oxoheptyl]tetrahydro-3,5-dimethyl-

α -Ethyl-6-[5-[2-(5-ethyltetrahydro-5-hydroxy-6-methyl-2H-pyran-2-yl)-15-hydroxy-2,10,12-trimethyl-1,6,8-trioxadispiro[4.1.5.3]pentadec-13-en-9-yl]-2-hydroxy-1,3-dimethyl-4-oxoheptyl]tetrahydro-3,5-dimethyl-2H-pyran-2-acetic acid [55134-13-9].

» Narasin Granular contains narasin mixed with suitable carriers and inactive ingredients prepared in a granular form that is free-flowing and free of aggregates. It contains not less than 100 mg and not more than 160 mg of narasin per g.

Packaging and storage—Preserve in well-closed containers. Avoid moisture and excessive heat.

Labeling—Label it to indicate that it is for animal use only. Label it also to indicate that it is for manufacturing, processing, or repackaging.

USP Reference standards (11)—

USP Monensin Sodium RS

USP Narasin RS

Identification—The retention time of the major peak for narasin A in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

Loss on drying (731)—Dry it in vacuum at 60° for 3 hours; it loses not more than 10% of its weight.

Powder fineness (811)—Not less than 99% passes a No. 30 sieve, and not more than 15% passes a No. 140 sieve.

Content of narasin A—Using the chromatogram of the *Assay preparation* obtained as directed in the *Assay*, calculate the percentage of narasin A by the formula:

$$100A / [A + (D + I)]$$

in which *A* is the narasin A biopotency and *D + I* is the narasin *D + I* biopotency. Not less than 85% of narasin A is found.

Assay—

Diluent—Prepare a mixture of methanol and water (9:1).

Mobile phase—Prepare a degassed mixture of methanol, water, and glacial acetic acid (94:6:0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Neutralized methanol—Add 1 g of sodium bicarbonate to 4 L of methanol, mix, and filter.

Derivatizing reagent—Dissolve 30 g of vanillin in a mixture of methanol and sulfuric acid (950:20) in a container protected from light. [Caution—To avoid splattering, add the sulfuric acid carefully and slowly with a pipet; do not pour. Allow the mixture of methanol and sulfuric acid to cool before adding the vanillin.] Do not filter.

Resolution solution—Prepare a solution in *Neutralized methanol* containing about 3 mg of USP Narasin RS and 1 mg of USP Monensin Sodium RS per mL. Transfer 2 mL of this solution to a 200-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Standard preparations—Dissolve an accurately weighed quantity of USP Narasin RS in *Neutralized methanol* to obtain a solution having a known concentration of about 1 mg per mL. Transfer 1.0 mL of this stock solution to a 200-mL volumetric flask, and transfer 2.0 mL and 4.0 mL of the stock solution to two separate 100-mL volumetric flasks, dilute each with *Diluent* to volume, and mix. These solutions con-

tain about 5, 20, and 40 μ g of USP Narasin RS per mL. Using the designated percentage of narasin A in the USP Narasin RS, calculate the exact narasin A concentration, in μ g per mL, in each of the *Standard preparations*.

Assay preparation—Transfer about 5 g of Narasin Granular, accurately weighed, to a suitable container, add 200.0 mL of *Diluent*, and shake by mechanical means for 1 hour. Allow the solids to settle, and quantitatively dilute an accurately measured volume of the supernatant with *Diluent* to obtain a solution containing about 20 μ g of narasin per mL. Pass a portion of this solution through a filter having a 0.5- μ m or finer porosity, and use the filtrate as the *Assay preparation*.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 4.6-mm \times 25-cm column that contains packing L1. The column outlet is attached to a tee, the opposing arm is attached to a tube from which is pumped the *Derivatizing reagent*, and the outlet is connected to a 2-mL postcolumn reaction coil maintained at 98°. The outlet of the reaction coil is connected to a detector set at 520 nm. The *Mobile phase* and the *Derivatizing reagent* flow at the rate of about 0.7 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for monensin B, 0.75 for monensin A, 1.0 for narasin A, and 1.1 for narasin *D + I*; and the resolution, *R*, between the monensin B peak and the monensin A peak is not less than 1.25, and between the monensin A peak and the narasin A peak not less than 3.5. Chromatograph the *Standard preparations*, and record the peak responses as directed for *Procedure*: the tailing factor for the narasin A peak is not more than 1.4 when calculated by the formula:

$$W_{0.1} / 2f$$

in which $W_{0.1}$ is the width of the peak at 10% of peak height; and *f* is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point on the baseline at which 10% peak height is reached. The relative standard deviation for replicate injections is not more than 10%. [NOTE—After use, flush the system with methanol.]

Procedure—Separately inject equal volumes (about 200 μ L) of the *Standard preparations* and the *Assay preparation* into the chromatograph, and measure the areas of the peak responses for the narasin A and narasin *D + I* peaks [NOTE—Narasin D and narasin I will co-elute under this chromatographic system.]

Plot the three narasin peak responses in the chromatograms obtained from the *Standard preparations* versus the concentration, in μ g per mL, of narasin A, and draw the straight line best fitting the three plotted points. From the graph so obtained, and the narasin A peak response in the chromatogram obtained from the *Assay preparation*, determine the concentration, C_A , in μ g per mL, of narasin A in the *Assay preparation*. From the same graph and the narasin *D + I* peak response in the chromatogram obtained from the *Assay preparation*, determine the concentration, C_{D+I} , in μ g per mL, of narasin *D + I* in the *Assay preparation*. Calculate the biopotency, in mg per g, in the portion of Narasin Granular taken by the formula:

$$(0.001)(C_A F_A + C_{D+I} F_{D+I})(VE / M)$$

in which F_A is 1.077 representing the biopotency conversion factor for narasin A; F_{D+I} is the biopotency conversion factor for narasin *D + I*; *V* is the extraction volume, in mL; *E* is the dilution factor used in diluting the extract to the final estimated concentration of 20 μ g per mL; and *M* is the weight, in g, of Narasin Granular taken to prepare the *Assay preparation*.

ration. Calculate the bioconversion factor, F_{D+I} , for narasin D + I by the formula:

$$(1.510D + 0.012I) / (D + I)$$

in which D and I are the specified percentages of narasin D and narasin I, respectively, in USP Narasin RS; 1.510 is the factor for converting narasin D to narasin D biopotency; and 0.012 is the factor for converting narasin I to narasin I biopotency.

Narasin Premix

» Narasin Premix contains Narasin Granular mixed with suitable diluents and inactive ingredients. It contains not less than 90 percent and not more than 110 percent of the labeled amount of narasin.

Packaging and storage—Preserve in well-closed containers. Avoid moisture and excessive heat.

Labeling—Label it to indicate that it is for animal use only. The label bears the statement, "Do not feed undiluted."

USP Reference standards (11)—

USP Monensin Sodium RS

USP Narasin RS

Identification—The retention time of the major peak for narasin A in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay*.

Loss on drying (731)—Dry it in vacuum at 60° for 3 hours: it loses not more than 12% of its weight.

Assay—

Diluent, Mobile phase, Neutralized methanol, Derivatizing reagent, Resolution solution, Standard preparations, and Chromatographic system—Proceed as directed in the *Assay* under *Narasin Granular*.

Assay preparation—Transfer about 5 g of Narasin Premix, accurately weighed, to a suitable container, add 200.0 mL of *Diluent*, and shake by mechanical means for 1 hour. Allow the solids to settle, and quantitatively dilute an accurately measured volume of the supernatant with *Diluent* to obtain a solution containing about 20 µg of narasin per mL. Pass a portion of this solution through a filter having a 0.5-µm or finer porosity, and use the filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Narasin Granular*. Calculate the biopotency, in mg per g, in the portion of Narasin Premix taken by the formula:

$$(0.001)(C_A F_A + C_{D+I} F_{D+I})(VE / M)$$

in which M is the weight, in g, of Narasin Premix taken to prepare the *Assay preparation*; and the other terms are as defined therein.

Naratriptan Tablets

» Naratriptan Tablets contain an amount of Naratriptan Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of naratriptan ($C_{17}H_{25}N_3O_2S$).

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.

USP Reference standards (11)—

USP Naratriptan Hydrochloride RS

USP Naratriptan Resolution Mixture RS

A mixture of naratriptan hydrochloride with approximately 0.1% each of naratriptan related compound A [3-(1-methylpiperidin-4-yl)-1H-indole hydrochloride] and naratriptan related compound B [2-[3-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-indole-5-yl]ethanesulfonic acid methylamide oxalate].

Identification—

A: Thin-Layer Chromatographic Identification Test (201)—

Diluent—Prepare a mixture of methylene chloride and methanol (1:1).

Adsorbent: high performance thin-layer chromatographic silica gel.

Test solution—Transfer a number of Tablets, equivalent to 5 mg of naratriptan, to a 25-mL flask, add 1.0 mL of water to wet the Tablets, and gently shake to remove the Tablet film coating. Add 4.5 mL of *Diluent*, and shake for 5 minutes or until the Tablets have dispersed. Centrifuge at 3000 rpm for 10 minutes, and pass through a nylon filter having a 0.45-µm porosity.

Developing solvent system: a mixture of methylene chloride, alcohol, and triethylamine (10:2:1).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 500 mL, deaerated.

Apparatus 1: 100 rpm.

Times: 15 minutes.

Procedure—Determine the amount of $C_{17}H_{25}N_3O_2S$ dissolved from the difference between first derivative absorbance values at the wavelengths of maximum and minimum in the range from 226 nm to 236 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard* solution having a known concentration of USP Naratriptan Hydrochloride RS in the same *Medium*. [NOTE—Do not sonicate the *Standard* solution to dissolve. Dissolve the USP Reference Standard with *Medium* at about 37°.]

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{17}H_{25}N_3O_2S$ is dissolved in 15 minutes.

Uniformity of dosage units (905): meet the requirements.

Chromatographic purity—

0.05 M Ammonium phosphate buffer and Resolution solution—Prepare as directed in the test for *Chromatographic purity* under *Naratriptan Hydrochloride*.

Solution A—Use filtered and degassed 0.05 M Ammonium phosphate buffer.

Solution B—Use filtered and degassed acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Test solution—Transfer 5 Tablets into a suitable amber flask. Add 20.0 mL of 0.1 N sodium hydroxide, and allow to stand for 10 minutes. Sonicate for 10 minutes with regular vigorous swirling of the flask. Add 30.0 mL of 0.05 M Ammonium phosphate buffer, and mix well. Centrifuge a portion of this solution at 3500 rpm for about 10 minutes, and pass through a suitable filter having a 0.45-µm porosity, discarding the first 3 mL of the filtrate.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The column temperature is maintained at 40°. The flow rate

is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–35.0	97→80	3→20	linear gradient
35.0–40.0	80	20	isocratic
40.0–41.0	80→97	20→3	linear gradient
41.0–51.0	97	3	re-equilibration

Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.07 for 2-[3-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-indol-5-yl]ethanesulfonic acid methylamide (naratriptan related compound B) and 1.0 for naratriptan; and the resolution, *R*, between naratriptan and naratriptan related compound B is not less than 1.5.

Procedure—Inject a volume (equivalent to about 5 µg of naratriptan hydrochloride) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for all of the peaks. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(r_i/F)/[r_N + \Sigma(r_i/F)]$$

in which *F* is the relative response factor (see the accompanying table for values) for each impurity; *r_i* is the peak response for each impurity; and *r_N* is the naratriptan peak response (see the accompanying table for limits). In addition to not exceeding the limits listed in the accompanying table, not more than 0.2% of any other individual impurity is found; and not more than 1.5% of total impurities is found.

Assay—

0.01 M Triethylamine phosphate buffer, Mobile phase, and Resolution solution—Prepare as directed in the Assay under Naratriptan Hydrochloride.

Standard preparation—Dissolve an accurately weighed quantity of USP Naratriptan Hydrochloride RS in 0.1 N sodium hydroxide to obtain a solution having a known concentration of about 0.2 mg per mL. Dilute an accurately measured volume of this solution in 0.01 M Triethylamine phosphate buffer to obtain a solution having a known concentration of about 20 µg per mL.

Assay preparation—Transfer 5 Tablets into an amber 250-mL volumetric flask, add 30 mL of 0.1 N sodium hydroxide, and shake on a wrist-action shaker for at least 30 minutes. Sonicate for 10 minutes with regular vigorous swirling of the flask. Add about 170 mL of 0.01 M Triethylamine phosphate buffer, and mix well. Allow to cool to room temperature, dilute with 0.01 M Triethylamine phosphate buffer to volume, and mix. Centrifuge a portion of this solution at 3500 rpm for about 10 minutes, and pass through a suitable filter having a 0.45-µm porosity, discarding the first 3 mL of the filtrate.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 224-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L11. The flow rate is about 1.3 mL per minute. Chromato-

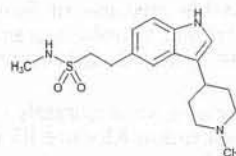
graph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for 3-(1-methylpiperidin-4-yl)-1H-indole (naratriptan related compound A), 1.0 for naratriptan, and 1.1 for naratriptan related compound B; and the resolution, *R*, between naratriptan related compound A and naratriptan and between naratriptan related compound B and naratriptan is not less than 1.5. Chromatograph the *Standard preparation*, record the chromatogram, and measure the peak response as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (equivalent to about 1 µg of naratriptan hydrochloride) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of naratriptan (C₁₇H₂₅N₃O₂S) in the portion of Tablets taken by the formula:

$$(335.47/371.93)100(C/D)(r_u/r_s)$$

in which 335.47 and 371.93 are the molecular weights of naratriptan and naratriptan hydrochloride, respectively; *C* is the concentration, in mg per mL, of USP Naratriptan Hydrochloride RS in the *Standard preparation*; *D* is the concentration, in mg per mL, of naratriptan in the *Assay preparation*, based upon the labeled quantity of naratriptan in the portion of Tablets taken and the extent of dilution; and *r_u* and *r_s* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Naratriptan Hydrochloride



C₁₇H₂₅N₃O₂S · HCl 371.93
1H-Indole-5-ethanesulfonamide, N-methyl-3-(1-methyl-4-piperidinyl)-, monohydrochloride.
N-Methyl-3-(1-methyl-4-piperidinyl)indole-5-ethanesulfonamide monohydrochloride
[143388-64-1].

» Naratriptan Hydrochloride contains not less than 98.0 percent and not more than 101.0 percent of C₁₇H₂₅N₃O₂S · HCl, calculated on the anhydrous and solvent free-basis.

Packaging and storage—Preserve in tight containers, and store below 30°.

Compound Name	Relative Retention Time	Relative Response Factor (F)	Limit (%)
2-[3-(1-Methyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-indol-5-yl]ethanesulfonic acid methylamide	about 1.07	0.6	0.2
2,2-Bis-[3-(1-methylpiperidin-4-yl)-1H-indol-5-yl]ethanesulfonic acid methylamide	about 1.26	0.6	0.2
1-Methyl-4-[5-(2-methylsulfamoyl-ethyl)-1H-indol-3-yl]-pyridinium chloride	about 1.33	0.4	0.3
2-[3-(1-methylpiperidin-4-yl)-5-(2-methylsulfamoyl-ethyl)-indol-1-yl]ethanesulfonic acid methylamide	about 1.44	0.6	0.2
4-[1,5-Bis-(2-methylsulfamoyl-ethyl)-1H-indol-3-yl]-1-methylpyridinium chloride	about 1.62	0.5	0.2

USP Reference standards (11)—

USP Naratriptan Hydrochloride RS

USP Naratriptan Resolution Mixture RS

A mixture of naratriptan hydrochloride with approximately 0.1% each of naratriptan related compound A [3-(1-methylpiperidin-4-yl)-1H-indole hydrochloride] and naratriptan related compound B [2-[3-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-indol-5-yl]ethanesulfonic acid methylamide oxalate].

NOTE—When performing assays and tests, store all standard, system suitability, and sample solutions in a cool place, protected from light.

Identification—

A: Infrared Absorption (197M).

B: The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

C: It meets the requirements of the test for dry chlorides under Chloride (191).

Water Determination, Method I (921): not more than 0.5%.

Delete the following:

• **Heavy metals, Method II (231):** 0.002%. • (Official 1-Jan-2018)

Chromatographic purity—

0.05 M Ammonium phosphate buffer—Dissolve 5.75 g of monobasic ammonium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 3.00 ± 0.05 .

Solution A—Prepare a filtered and degassed mixture of 0.05 M Ammonium phosphate buffer and acetonitrile (97:3).

Solution B—Prepare a filtered and degassed mixture of 0.05 M Ammonium phosphate buffer and acetonitrile (4:1).

Mobile phase—Use variable mixtures of Solution A and Solution B as directed for Chromatographic system. Make adjustments if necessary (see System Suitability under Chromatography (621)).

Resolution solution—Dissolve an accurately weighed quantity of USP Naratriptan Resolution Mixture RS in water to obtain a solution having a known concentration of about 0.11 mg per mL.

Test solution—Dissolve an accurately weighed quantity of Naratriptan Hydrochloride in water to obtain a solution having a known concentration of about 0.11 mg per mL.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 225-nm detector and a 4.6-mm \times 15-cm column that contains 4- μ m packing L1. The column temperature is maintained at 40°. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–35.0	100→0	0→100	linear gradient
35.0–40.0	0	100	isocratic
40.0–41.0	0→100	100→0	linear gradient
41.0–51.0	100	0	re-equilibration

Chromatograph the Resolution solution, and record the peak responses as directed for Procedure: the relative retention times are about 1.04 for 2-[3-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-indol-5-yl]ethanesulfonic acid methylamide (naratriptan related compound B) and 1.0 for naratriptan; and the resolution, R , between naratriptan and naratriptan related compound B is not less than 1.5.

Procedure—Inject a volume (about 20 μ L) of the Test solution into the chromatograph, record the chromatogram, and measure the areas for all of the peaks. Calculate the percentage of each impurity in the portion of Naratriptan Hydrochloride taken by the formula:

$$100(r_i/F)/[r_N + \Sigma(r_i/F)]$$

in which F is the relative response factor (see the accompanying table for values) for each impurity; r_i is the peak response for each impurity; and r_N is the naratriptan peak response (see the accompanying table for limits). In addition to not exceeding the limits listed in the accompanying table, not more than 0.1% of any other individual impurity is found; and not more than 1.5% of total impurities is found.

Assay—

0.01 M Triethylamine phosphate buffer—Dilute 0.6 mL of phosphoric acid with water to 900 mL, and adjust with triethylamine to a pH of 2.5.

Mobile phase—Prepare a filtered and degassed mixture of 0.01 M Triethylamine phosphate buffer and isopropyl alcohol (9:1). Make adjustments if necessary (see System Suitability under Chromatography (621)).

System suitability preparation—Dissolve an accurately weighed quantity of USP Naratriptan Resolution Mixture RS in Mobile phase to obtain a solution having a concentration of about 0.7 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Naratriptan Hydrochloride RS in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 0.11 mg per mL.

Assay preparation—Transfer about 11 mg of Naratriptan Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 282-nm detector and a 4.6-mm \times 15-cm column that contains 3- μ m packing

Compound Name	Relative Retention Time	Relative Response Factor (F)	Limit (%)
3-(1-Methylpiperidin-4-yl)-1H-indole	about 0.93	1.0	0.2
2-[3-(1-Methyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-indol-5-yl]ethanesulfonic acid methylamide	about 1.04	0.6	0.1
2,2-Bis-[3-(1-methylpiperidin-4-yl)-1H-indol-5-yl]ethanesulfonic acid methylamide	about 1.18	0.6	0.2
1-Methyl-4-[5-(2-methylsulfamoyl-ethyl)-1H-indol-3-yl]-pyridinium chloride	about 1.25	0.4	0.2
2-[3-(1-Methylpiperidin-4-yl)-5-(2-methylsulfamoyl-ethyl)-indol-1-yl]ethanesulfonic acid methylamide	about 1.36	0.6	0.3
4-[1,5-Bis-(2-methylsulfamoyl-ethyl)-1H-indol-3-yl]-1-methylpyridinium chloride	about 1.44	0.5	0.1
2-[3-(1-Methylpiperidin-4-yl)-1H-indol-5-yl]ethane-sulfonic acid methyl-(2-methylsulfamoyl-ethyl)amide	about 1.48	1.0	0.2
5-Ethyl-3-(1-methylpiperidin-4-yl)-1H-indole	about 1.90	1.00	0.2

L11. The column temperature is maintained at 35°. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution, R , between naratriptan related compound A and naratriptan and between naratriptan related compound B and naratriptan is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%. [NOTE—For identification purposes, the approximate relative retention times are about 0.9 for naratriptan related compound A, 1.0 for naratriptan, and 1.1 for naratriptan related compound B.]

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{17}H_{25}N_3O_2S \cdot HCl$ in the portion of Naratriptan Hydrochloride taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Naratriptan Hydrochloride RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Naratriptan Compounded Oral Suspension

DEFINITION

Naratriptan Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of naratriptan ($C_{17}H_{25}N_3O_2S$). Prepare Naratriptan Compounded Oral Suspension containing 0.5 mg/mL of Naratriptan as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Naratriptan (as Naratriptan Hydrochloride)	50 mg (55.43 mg)
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, NF (regular or sugar-free), and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. If using tablets, place the required number of tablets in a suitable mortar, and comminute the tablets to a fine powder or add Naratriptan Hydrochloride powder. Add the Vehicle in small portions and triturate to make a smooth paste. Add increasing volumes of the Vehicle to make a naratriptan suspension that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the Vehicle to bring to final volume, and mix well.

ASSAY

PROCEDURE

Mobile phase: 2-Propanol and 12 mM triethylamine phosphate buffer (1:10). Make adjustments if necessary.

Standard stock solution: 0.5 mg/mL of USP Naratriptan Hydrochloride RS in *Mobile phase*

Standard solution: Transfer 1.0 mL of *Standard stock solution* to a 25-mL volumetric flask, dilute with *Mobile phase* to volume to obtain a solution containing 20 μ g/mL of naratriptan hydrochloride, and pass through a suitable filter of 0.22- μ m pore size.

Sample solution: Shake the Oral Suspension thoroughly by hand. Pipet 0.4 mL into a 10-mL volumetric flask. Add 1 mL of 0.1 N sodium hydroxide solution by

pipet, and sonicate for 5 min. Dilute with *Mobile phase* to volume, and mix to obtain a nominal concentration of 20 μ g/mL of naratriptan. Centrifuge, and pass the naratriptan solution through a suitable filter of 0.22- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L11

Flow rate: 1.4 mL/min

Injection volume: 25 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Retention time: 9.7 min for the naratriptan peak

Relative standard deviation: NMT 4.9% for the replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of naratriptan ($C_{17}H_{25}N_3O_2S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Naratriptan Hydrochloride RS in the *Standard solution* (μ g/mL), on the anhydrous basis

C_U = nominal concentration of naratriptan in the *Sample solution* (μ g/mL)

M_{r1} = molecular weight of naratriptan, 335.47

M_{r2} = molecular weight of naratriptan hydrochloride, 371.93

Acceptance criteria: 90.0%–110.0%

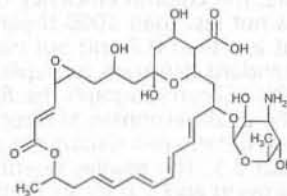
SPECIFIC TESTS

- pH (791):** 4.0–4.5

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- BEYOND-USE DATE:** NMT 7 days after the date on which it was compounded when stored at controlled room temperature, and NMT 90 days after the date on which it was compounded when stored in a refrigerator
- LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- USP REFERENCE STANDARDS (11)**
USP Naratriptan Hydrochloride RS

Natamycin



$C_{33}H_{47}NO_{13}$ 665.73

Stereoisomer of 22-[(3-amino-3,6-dideoxy- β -D-mannopyranosyl)oxy]-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo [22.3.1.0^{5,7}]octacos-8,14,16,18,20-pentaene-25-carboxylic acid [7681-93-8].

» Natamycin contains not less than 90.0 percent and not more than 102.0 percent of $C_{33}H_{47}NO_{13}$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Natamycin RS

Identification—Transfer 50 mg, accurately weighed, to a 200-mL volumetric flask, add 5.0 mL of water, and moisten the specimen. Add 100 mL of a 1 in 1000 solution of glacial acetic acid in methanol, and shake by mechanical means in the dark until dissolved. Dilute with the acetic acid-methanol solution to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with the acetic acid-methanol solution to volume, and mix: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Natamycin RS, concomitantly measured.

Crystallinity (695): meets the requirements.

pH (791): between 5.0 and 7.5, in an aqueous suspension containing 10 mg per mL.

Water Determination, Method I (921): between 6.0% and 9.0%.

Assay—[NOTE—Throughout the Assay, protect from direct light all solutions containing natamycin.]

Mobile phase—Dissolve 3.0 g of ammonium acetate and 1.0 g of ammonium chloride in 760 mL of water, and mix. Add 5.0 mL of tetrahydrofuran and 240 mL of acetonitrile, and mix. Pass this solution through a 0.5 μ m or finer porosity filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Transfer about 20 mg of USP Natamycin RS, accurately weighed, to a 100-mL volumetric flask. Add 5.0 mL of tetrahydrofuran, and sonicate for 10 minutes. Add 60 mL of methanol, and swirl to dissolve. Add 25 mL of water, and mix. Allow to cool to room temperature. Dilute with water to volume, and mix. Pass this solution through a suitable membrane filter of 0.5 μ m or finer porosity.

Resolution solution—Dissolve 20 mg of Natamycin in a mixture of 99 mL of methanol and 1 mL of 0.1 N hydrochloric acid, and allow to stand for 2 hours. [NOTE—Use this solution within 1 hour.]

Assay preparation—Transfer about 20 mg of Natamycin, accurately weighed, to a 100-mL volumetric flask. Proceed as directed under *Standard preparation*, beginning with "add 5.0 mL of tetrahydrofuran."

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 303-nm detector and a 4.6-mm \times 25-cm analytical column that contains packing L1. [NOTE—A 3.9-mm \times 20-mm pre-column may be used to extend the useful life of the analytical column.] The flow rate is about 3 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 3000 theoretical plates, the tailing factor is not less than 0.8 and not more than 1.3, and the relative standard deviation for replicate injections is not more than 1.0%. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, R , between natamycin and its methyl ester is not less than 2.5. The relative retention times are about 0.7 for natamycin and 1.0 for its methyl ester.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the per-

centage of natamycin ($C_{33}H_{47}NO_{13}$) in the portion of Natamycin taken by the formula:

$$0.1(W_S P_S / W_U)(r_U / r_S)$$

in which W_S is the weight, in mg, of USP Natamycin RS taken to prepare the *Standard preparation*; P_S is the stated content, in μ g per mg, of USP Natamycin RS; W_U is the weight, in mg, of Natamycin taken to prepare the *Assay preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Natamycin Ophthalmic Suspension

» Natamycin Ophthalmic Suspension is a sterile suspension of Natamycin in a suitable aqueous vehicle. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of $C_{33}H_{47}NO_{13}$. It contains one or more suitable preservatives.

Packaging and storage—Preserve in tight, light-resistant containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards (11)—

USP Natamycin RS

Identification—Transfer a volume of Ophthalmic Suspension, equivalent to about 50 mg of natamycin, to a 200-mL volumetric flask, and add water to make a volume of 5 mL. Proceed as directed in the *Identification* test under *Natamycin*, beginning with "Add 100 mL of a 1 in 1000 solution of glacial acetic acid in methanol:" the specified result is obtained.

Sterility Tests (71)—It meets the requirements as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, using 0.25 mL of the Ophthalmic Suspension taken from each container.

pH (791): between 5.0 and 7.5.

Assay—[NOTE—Throughout the Assay protect from direct light all solutions containing natamycin.]

Mobile phase, Standard preparation, Resolution solution, and Chromatographic system—Proceed as directed in the Assay under *Natamycin*.

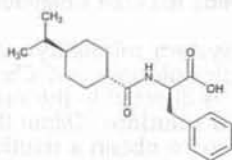
Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, equivalent to about 50 mg of natamycin, to a 250-mL volumetric flask. Add 12.5 mL of tetrahydrofuran, and sonicate for 10 minutes. Add 150 mL of methanol, and swirl to dissolve. Add 60 mL of water, and mix. Allow to cool to room temperature. Dilute with water to volume, and mix. Filter this solution through a suitable membrane filter of 0.5- μ m or finer porosity.

Procedure—Proceed as directed in the Assay under *Natamycin*. Calculate the quantity, in mg, of $C_{33}H_{47}NO_{13}$ in each mL of the Ophthalmic Suspension taken by the formula:

$$0.25C(P_S / V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Natamycin RS in the *Standard preparation*; P_S is the stated content, in μ g per mg, of USP Natamycin RS; V is the volume, in mL, of Ophthalmic Suspension taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nateglinide



$C_{19}H_{27}NO_3$ 317.42
D-Phenylalanine, N-[[trans-4-(1-methylethyl)cyclohexyl]carbonyl]-;
(-)-N-[[trans-4-Isopropylcyclohexyl]carbonyl]-D-phenylalanine
[105816-04-4].

DEFINITION

Nateglinide contains NLT 98.0% and NMT 102.0% of $C_{19}H_{27}NO_3$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer: 8.5 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 7.5.

Mobile phase: Methanol and *Buffer* (1:1)

System suitability stock solution: 0.2 mg/mL each of USP Nateglinide Related Compound C RS and DL-phenylalanine in methanol. [NOTE—Sonicate, if necessary.]

System suitability solution: Transfer USP Nateglinide RS to a suitable volumetric flask, dissolve first in methanol, using 45% of the final volume, add *System suitability stock solution* equal to 5% of the final volume, and then dilute with *Buffer* to volume to obtain a solution containing about 1.0 mg/mL of nateglinide and about 0.01 mg/mL each of nateglinide related compound C and DL-phenylalanine.

Standard solution: 1.0 mg/mL of nateglinide prepared as follows: transfer USP Nateglinide RS to a suitable volumetric flask, dissolve first in methanol, using 50% of the final volume, and then dilute with *Buffer* to volume.

Sample solution: Transfer about 100 mg of Nateglinide to a 100-mL volumetric flask, dissolve in 50 mL of methanol, and dilute with *Buffer* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 6-mm × 15-cm; 6-μm packing L71 (see *Chromatographic Reagents under Reagents, Indicators, and Solutions*)

Column temperature: 30°

Flow rate: 1 mL/min

Injection size: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 0.9 between nateglinide related compound C and nateglinide, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of nateglinide ($C_{19}H_{27}NO_3$) in the portion of Nateglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Nateglinide RS in the *Standard solution* (mg/mL)
 C_U = concentration of Nateglinide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 10 ppm • (Official 1-Jan-2018)

• LIMIT OF NATEGLINIDE RELATED COMPOUND A AND OTHER IMPURITIES

Buffer: 7.8 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Buffer* (7:13)

System suitability stock solution: Dissolve USP Nateglinide Related Compound A RS in acetonitrile to obtain a solution containing about 0.6 mg/mL. Further dilute this solution with *Mobile phase* to obtain a solution containing about 0.12 mg/mL.

System suitability solution: Transfer an amount of USP Nateglinide RS to a suitable volumetric flask, dissolve first in acetonitrile using 10% of the final volume, then add *System suitability stock solution* equal to 10% of the final volume, and dilute with *Mobile phase* to volume to obtain a solution containing about 6 mg/mL of nateglinide and about 0.012 mg/mL of nateglinide related compound A.

Standard solution: Dissolve USP Nateglinide RS in acetonitrile to obtain a solution having a known concentration of about 0.3 mg/mL. Further dilute this solution with *Mobile phase* to obtain a solution having a known concentration of about 0.06 mg/mL.

Sample solution: Transfer 60 mg of Nateglinide to a 10-mL volumetric flask, dissolve in a minimal amount of acetonitrile, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 5-cm; 5-μm packing L7

Column temperature: 40°

Flow rate: 2 mL/min

Injection size: 100 μL

Run time: 5 times the retention time of nateglinide

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.5 between nateglinide related compound A and nateglinide, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Nateglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- r_U = peak response of each impurity from the *Sample solution*
 r_S = peak response of nateglinide from the *Standard solution*
 C_S = concentration of USP Nateglinide RS in the *Standard solution* (mg/mL)
 C_U = concentration of Nateglinide in the *Sample solution* (mg/mL)

F = relative response factor (see Table 1)
 Acceptance criteria
 Individual impurities: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Nateglinide related compound A ^a	0.5	0.015	0.2
Ethyl analog ^b	0.6	1.0	0.1
Nateglinide	1.0	—	—
IPP impurity ^c	3.1	1.0	0.1
Ester impurity ^d	4.1	0.94	0.1
Any other individual impurity	—	1.0	0.1

^a *trans*-4-Isopropylcyclohexylcarboxylic acid.

^b *N*-(*trans*-4-Ethylcyclohexylcarbonyl)-D-phenylalanine.

^c *N*-(*trans*-4-Isopropylcyclohexylcarbonyl)-D-phenylalanine-D-phenylalanine.

^d *N*-(*trans*-4-isopropylcyclohexylcarbonyl)-D-phenylalanine-ethyl ester.

• LIMIT OF NATEGLINIDE RELATED COMPOUND B

Mobile phase: 0.77 g/L of ammonium acetate in methanol.

[NOTE—The following solutions are stable for up to 48 h when stored in a refrigerator.]

System suitability solution: 10 mg/mL of USP Nateglinide RS and 0.02 mg/mL of USP Nateglinide Related Compound B RS in methanol

Standard solution: 0.02 mg/mL of USP Nateglinide Related Compound B RS in methanol. [NOTE—Nateglinide related compound B is *N*-(*trans*-4-isopropyl-cyclohexyl-carbonyl)-L-phenylalanine.]

Sample solution: 10 mg/mL of Nateglinide in methanol

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 4-mm × 25-cm or 4.6-mm × 25-cm; 5-μm packing L72 (see Chromatographic Reagents under Reagents, Indicators, and Solutions)

Column temperature: 40°

Flow rate: 0.8 mL/min. [NOTE—The flow rate can be adjusted as needed to achieve a recommended retention time of nateglinide related compound B at about 25 min.]

Injection size: 10 μL

System suitability

[NOTE—The elution order is nateglinide related compound B, followed by the nateglinide peak.]

Samples: System suitability solution and Standard solution

Suitability requirements

Resolution: NLT 0.8 between nateglinide related compound B and nateglinide, System suitability solution

Relative standard deviation: NMT 5%, Standard solution

Analysis

Samples: Standard solution and Sample solution
 Calculate the percentage of nateglinide related compound B in the portion of Nateglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nateglinide related compound B from the Sample solution

r_S = peak response of nateglinide related compound B from the Standard solution

C_S = concentration of USP Nateglinide Related Compound B RS in the Standard solution (mg/mL)

C_U = concentration of Nateglinide in the Sample solution (mg/mL)

Acceptance criteria: NMT 0.2%

• LIMIT OF NATEGLINIDE RELATED COMPOUND C AND PHENYLALANINE

Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Diluted standard solution: Dilute the Standard solution with Mobile phase to obtain a solution having a known concentration of about 0.01 mg/mL of nateglinide.

Analysis

Samples: Sample solution and Diluted standard solution

Calculate the percentage of each specified impurity listed in Table 2 in the portion of Nateglinide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the Sample solution

r_S = peak response of nateglinide from the Diluted standard solution

C_S = concentration of nateglinide in the Diluted standard solution (mg/mL)

C_U = concentration of Nateglinide in the Sample solution (mg/mL)

F = relative response factor of each individual impurity (see Table 2)

Acceptance criteria

Individual impurities: See Table 2.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Phenylalanine	0.2	1.5	0.2
Nateglinide <i>cis</i> -isomer ^a (related compound C)	0.9	0.97	0.2
Nateglinide	1.0	—	—

^a *N*-(*cis*-4-isopropylcyclohexylcarbonyl)-D-phenylalanine.

Total impurities: The sum of all impurities found in the tests for Limit of Nateglinide Related Compound A and Other Impurities, Limit of Nateglinide Related Compound B, and Limit of Nateglinide Related Compound C and Phenylalanine is NMT 0.5%.

SPECIFIC TESTS

- **LOSS ON DRYING (731):** Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.

• USP REFERENCE STANDARDS (11)

USP Nateglinide RS

(-)-*N*-[(*trans*-4-Isopropylcyclohexyl)carbonyl]-D-phenylalanine.

$C_{19}H_{27}NO_3$ 317.42

USP Nateglinide Related Compound A RS
trans-4-Isopropylcyclohexylcarboxylic acid.

$C_{10}H_{18}O_2$ 170.2

USP Nateglinide Related Compound B RS

N-(*trans*-4-Isopropylcyclohexylcarbonyl)-L-phenylalanine.

$C_{19}H_{27}NO_3$ 317.4

USP Nateglinide Related Compound C RS

Nateglinide *cis*-isomer, *N*-(*cis*-4-isopropylcyclohexyl-carbonyl)-D-phenylalanine.

$C_{19}H_{27}NO_3$ 317.4

Nateglinide Tablets

DEFINITION

Nateglinide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of nateglinide ($C_{19}H_{27}NO_3$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Diluent: Acetonitrile and water (11:9)

Mobile phase: Acetonitrile and 0.05% solution of trifluoroacetic acid (23:27)

Standard solution: 0.72 mg/mL of USP Nateglinide RS prepared as follows. Transfer USP Nateglinide RS to a suitable volumetric flask, and add acetonitrile to 40% of the volume of the flask. [NOTE—Sonicate to dissolve.] Add water equivalent to 30% of the final volume, mix, cool the solution to room temperature, and dilute with *Diluent* to volume.

Sample solution: Place 20 Tablets into a 500-mL volumetric flask, and add 60 mL of water to disintegrate the Tablets. [NOTE—Sonicate with cooling, if necessary.] Add 280 mL of acetonitrile, and shake by mechanical means for at least 30 min. Dilute with *Diluent* to volume. Pass a portion through a 0.45- μ m glass microfiber filter, discarding the first 10 mL of the filtrate, or use centrifugation to obtain a clear solution. Dilute an aliquot of this solution with *Diluent* to obtain a solution having a concentration of 0.72 mg/mL based on the label claim.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1.5 mL/min

Injection size: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.8

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled quantity of $C_{19}H_{27}NO_3$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: 0.01 N hydrochloric acid containing 0.5% (w/v) of sodium lauryl sulfate; 1000 mL

Apparatus 2: 50 rpm

Time: 30 min

Determine the quantity of $C_{19}H_{27}NO_3$ dissolved by employing the following method.

Solution A: 6.9 mg/mL of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and *Solution A* (45:55)

Standard stock solution: 0.3 mg/mL of USP Nateglinide RS prepared as follows. Transfer USP Nateglinide

RS to a suitable volumetric flask, dissolve in a small volume of acetonitrile not exceeding 5% of the final volume, and dilute with *Medium* to volume.

Standard solution: 0.12 mg/mL of USP Nateglinide RS in *Medium*, from the *Standard stock solution*

Sample solution: Pass through a suitable filter with pore size of 0.7 μ m.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 5-cm; 5- μ m packing L1

Flow rate: 1.5 mL/min

Injection size: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{19}H_{27}NO_3$ dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times V) \times (100/L)$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = volume of *Medium*, 1000 mL

L = label claim (mg/Tablet)

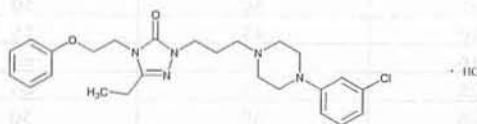
Tolerances: NLT 80% (Q) of the labeled amount of $C_{19}H_{27}NO_3$ is dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
USP Nateglinide RS

Nefazodone Hydrochloride



$C_{25}H_{32}ClN_5O_2 \cdot HCl$

506.47

3H-1,2,4-Triazol-3-one, 2-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-ethyl-2,4-dihydro-4-(2-phenoxyethyl)-, monohydrochloride;

1-[3-[4-(*m*-Chlorophenyl)-1-piperazinyl]propyl]-3-ethyl-4-(2-phenoxyethyl)- Δ^2 -1,2,4-triazolin-5-one monohydrochloride [82752-99-6].

DEFINITION

Nefazodone Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{25}H_{32}ClN_5O_2 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

- A. INFRARED ABSORPTION (197K)**

- B. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements

Sample solution: 10 mg/mL in methanol

ASSAY

PROCEDURE

Sample solution: 800 mg of Nefazodone Hydrochloride in 50 mL of glacial acetic acid. Add 15 mL of 3% mercuric acetate in glacial acetic acid.

Titrimetric system

(See *Titrimetry* (541).)

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis

Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Carry out a blank titration.

Calculate the percentage of $C_{25}H_{32}ClN_5O_2 \cdot HCl$ in the portion of Nefazodone Hydrochloride taken:

$$\text{Result} = ((V - B) \times N \times F/W) \times 100$$

V = sample titrant volume (mL)

B = blank titrant volume (mL)

N = titrant normality (mEq/mL)

F = 506.5 (mg/mEq)

W = sample weight (mg)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Inorganic Impurities

• **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

• **HEAVY METALS**, Method II (231): NMT 10 ppm (Official 1-Jan-2018)

Organic Impurities

PROCEDURE

Diluent: Acetonitrile and water (1:1)

Solution A: 0.77 g/L of ammonium acetate in water. Adjust with triethylamine to a pH of 7.10 ± 0.05 , and degas.

Solution B: Acetonitrile (degassed)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	50	50
10	45	55
16	35	65
25	35	65
26	50	50
35	50	50

Impurities stock solution: 0.1 mg/mL of USP Nefazodone Related Compound A RS and USP Nefazodone Related Compound B RS in Diluent

Standard stock solution: 0.1 mg/mL of USP Nefazodone Hydrochloride RS in Diluent

System suitability solution: 5 µg/mL each of USP Nefazodone Related Compound A RS and USP Nefazodone Related Compound B RS from the Impurities stock solution in the Standard stock solution

Standard solution: 1 µg/mL each of Nefazodone Hydrochloride, USP Nefazodone Related Compound A RS, and USP Nefazodone Related Compound B RS from the Standard stock solution and the Impurities stock solution in Diluent

Sample solution: 1 mg/mL of Nefazodone Hydrochloride in Diluent

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.7 mL/min

Injection size: 10 µL

System suitability

Samples: System suitability solution and Standard solution

Suitability requirements

Resolution: NLT 4.0 between nefazodone related compound A and nefazodone hydrochloride; NLT 1.5 between nefazodone hydrochloride and nefazodone related compound B, *System suitability solution*

Relative standard deviation: NMT 5.0% for nefazodone related compound A and nefazodone related compound B, *Standard solution*

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each nefazodone related compound in the portion of Nefazodone Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak area of the corresponding nefazodone related compound from the *Sample solution*

r_s = peak area of the corresponding nefazodone related compound from the *Standard solution*

C_s = concentration of the relevant USP RS in the *Standard solution* (mg/mL)

C_u = concentration of the *Sample solution* (mg/mL)

[NOTE—Use the peak area for nefazodone hydrochloride in the *Standard solution* as r_s to calculate any unknown impurity.]

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nefazodone related compound A	1.2	0.2
Nefazodone related compound B	0.94	0.2
Nefazodone	1.0	—
Any other individual, unidentified impurity	—	0.1

SPECIFIC TESTS

• **LOSS ON DRYING** (731): Dry a sample in a vacuum at 105° for 3 h; it loses NMT 0.5% of its weight.

• **COMPLETENESS OF SOLUTION** (641): Meets the requirements

Sample solution: 25 mg/mL in methanol

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE**: Preserve in tight containers. Store between 15° and 30°.

• **USP REFERENCE STANDARDS** (11)

USP Nefazodone Hydrochloride RS

USP Nefazodone Related Compound A RS

1-(3-Chloropropyl)-4-(chlorophenyl)piperazine.

$C_{13}H_{18}Cl_2N_2$ 273.20

USP Nefazodone Related Compound B RS

2-(3-(4-(Chlorophenyl)-1-piperazinyl)propyl)-5-ethyl-2,4-dihydro-4-(2-phenoxyethyl)-3H-1,2,4-triazol-3-one.

$C_{25}H_{32}ClN_5O_2$ 470.01

Nefazodone Hydrochloride Tablets

» Nefazodone Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nefazodone hydrochloride ($C_{25}H_{32}ClN_5O_2 \cdot HCl$).

Packaging and storage—Preserve in tight containers. Store at controlled room temperature.

USP Reference standards (11)—

USP Nefazodone Hydrochloride RS

USP Nefazodone Related Compound A RS

1-(3-Chloropropyl)-4-(chlorophenyl)piperazine.

$C_{13}H_{18}Cl_2N_2$ 273.20

USP Nefazodone Related Compound B RS

2-(3-(4-(Chlorophenyl)-1-piperazinyl)propyl)-5-ethyl-2,4-dihydro-4-(2-phenoxyethyl)-3H-1,2,4-triazol-3-one.

$C_{25}H_{32}ClN_5O_2$ 470.01

Identification—

A: Infrared Absorption (197K).

B: The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL, deaerated.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Standard stock solution—Transfer about 70 mg of USP Nefazodone Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask. Add 2.5 mL of methanol, dilute with Medium to volume, and mix.

Standard solution—Dilute the Standard stock solution with Medium in such a way that the final concentration is similar to the one expected in the Test solution.

Test solution—Use portions of the solution under test passed through a 0.45- μ m PVDF filter, discarding the first 5 mL.

Procedure—Determine the percentage of the labeled amount of nefazodone hydrochloride dissolved by employing UV absorption, using a suitable spectrophotometer, at the wavelength of maximum absorbance at about 246 nm, on the Test solution in comparison with the Standard solution, using Medium as the blank. Calculate the percentage of nefazodone hydrochloride ($C_{25}H_{32}ClN_5O_2 \cdot HCl$) dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times LC}$$

in which A_U and A_S are the absorbances obtained from the Test solution and the Standard solution, respectively; C_S is the concentration, in mg per mL, of USP Nefazodone Hydrochloride RS in the Standard solution; 900 is the volume, in mL, of Medium; 100 is the conversion factor to percentage; and LC is the tablet label claim, in mg.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{25}H_{32}ClN_5O_2 \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Related compounds—

Dilute acetic acid, Buffer solution, and Mobile phase—Proceed as directed in the Assay.

Nefazodone related compound A stock solution—Prepare a solution of USP Nefazodone Related Compound A RS in Mobile phase having a known concentration of about 80 μ g per mL.

Nefazodone related compound B stock solution—Prepare a solution of USP Nefazodone Related Compound B RS in Mobile phase having a known concentration of about 80 μ g per mL.

System suitability solution—Transfer about 10 mg of USP Nefazodone Hydrochloride RS into a 10-mL volumetric flask. Add 2.0 mL of Nefazodone related compound A stock solution and 2.0 mL of Nefazodone related compound B stock solution, and mix to dissolve the nefazodone hydrochloride. Dilute with Mobile phase to volume, and mix.

Standard solution—Use the Standard preparation, prepared as directed in the Assay.

Test solution—Use the Assay stock preparation, prepared as directed in the Assay.

Chromatographic system—Prepare as directed in the Assay. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure. Identify the peaks using the relative retention times given in Table 1: the resolution, R , between nefazodone related compound A and nefazodone hydrochloride is not less than 2.0; and the resolution, R , between nefazodone related compound B and nefazodone hydrochloride is not less than 1.5. [NOTE—Approximate relative retention times are provided in Table 1 for informational purposes only.]

Procedure—Inject equal volumes (about 10 μ L) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of individual nefazodone related compounds in the portion of Tablets taken by the formula:

$$100(r_U / r_S)(C_S / C_T)(1/F)$$

in which r_U is the individual peak response for each nefazodone related compound obtained from the Test solution; r_S is the response of the corresponding peak in the Standard solution, respectively; C_S and C_T are the concentrations, in mg per mL, of nefazodone hydrochloride in the Standard solution and the Test solution, respectively; and F is the relative response factor obtained from Table 1. The related compound requirements are listed in Table 1.

Table 1

Related Compound	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Nefazodone related compound A	1.4	1.2	0.2
Nefazodone related compound B	0.9	1.0	0.2
Any individual unknown impurity	—	1.0	0.2 each
Total known and unknown	—	—	0.5

Assay—

Dilute acetic acid—Prepare a mixture of acetic acid and water (1:1).

Buffer solution—Dissolve 0.77 g of ammonium acetate in 1 L of water. Add 1.0 mL of triethylamine, and mix well. Adjust with Dilute acetic acid to a pH of 7.10 ± 0.05 , and mix.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and Buffer solution (58:42). Make adjustments if necessary (see System Suitability under Chromatography (621)).

Standard preparation—Prepare a solution of USP Nefazodone Hydrochloride RS in Mobile phase having a known concentration of about 0.1 mg per mL.

Assay stock preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed por-

tion of the powder, equivalent to about 250 mg of nefazodone hydrochloride, based on the label claim, to a 250-mL volumetric flask, add about 125 mL of *Mobile phase*, and sonicate for about 10 minutes with occasional shaking. Dilute with *Mobile phase* to volume, and mix to obtain a solution having a concentration of about 1 mg per mL of nefazodone hydrochloride. Pass a portion of this solution through a filter having a 0.45- μ m or finer porosity, and use the filtrate, which has a concentration of about 1 mg per mL of nefazodone hydrochloride.

Assay preparation—Transfer 5.0 mL of *Assay stock preparation* into a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix to obtain a solution having a concentration of 0.1 mg per mL of nefazodone hydrochloride.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 250-nm detector and a 4.6-mm \times 25-cm column containing 5- μ m L1 packing. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 30°. Inject the *Standard preparation*, and record the peak responses as directed for *Procedure*; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in percent of label claim, of nefazodone hydrochloride ($C_{25}H_{32}ClN_5O_2 \cdot HCl$) in the portion of Tablets taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of nefazodone hydrochloride in the *Standard preparation* and the *Assay preparation*, respectively; and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin for Injection

» Neomycin for Injection contains an amount of Neomycin Sulfate equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of neomycin.

Change to read:

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

USP Reference standards (11)—

USP Endotoxin RS
USP Neomycin Sulfate RS

Thin-layer chromatographic identification test (201BNP): meets the requirements.

Bacterial Endotoxins Test (85)—It contains not more than 1.30 USP Endotoxin Units per mg of neomycin.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

Other requirements—It meets the requirements for *pH and Loss on drying under Neomycin Sulfate* and for *Uniformity of Dosage Units* (905) and *Labeling* (7), *Labels and Labeling for Injectable Products*.

Change to read:

Assay—

Assay preparation 1 (where it is packaged for dispensing)—Constitute Neomycin for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Buffer B.3* (CN 1-May-2017) to obtain a solution having a convenient concentration.

Assay preparation 2 (where it is packaged for dispensing and where the labeling states the quantity of neomycin in a given volume of constituted solution)—Constitute Neomycin for Injection as directed in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Buffer B.3* (CN 1-May-2017) to obtain a solution having a convenient concentration.

Procedure—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with *Buffer B.3* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin Boluses

» Neomycin Boluses contain an amount of Neomycin Sulfate equivalent to not less than 90.0 percent and not more than 125.0 percent of the labeled amount of neomycin.

Packaging and storage—Preserve in tight containers.

Labeling—Label Boluses to indicate that they are for veterinary use only.

USP Reference standards (11)—

USP Neomycin Sulfate RS

Identification—Blend a Bolus with 250 mL of water. Filter a portion of the suspension obtained. If necessary, dilute a portion of the filtrate with water to obtain a test solution containing about 2 mg of neomycin per mL. Dissolve a quantity of USP Neomycin Sulfate RS in water to obtain a Standard solution containing about 2 mg of neomycin per mL. Separately apply 1 μ L of each solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of water, butyl alcohol, glacial acetic acid, and pyridine (35:30:22:6) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry at about 110° for about 5 minutes. Spray the plate evenly with a solution of ninhydrin (2 mg per mL), and dry the plate at about 100° for about 5 minutes. Locate the spots on the plate: the R_f value of the principal spot in the chromatogram obtained from the test solution corresponds to that of the principal spot in the chromatogram obtained from the Standard solution.

Uniformity of dosage units (905): meet the requirements for *Weight Variation*.

Disintegration (701): 60 minutes.

Change to read:

Assay—Proceed as directed for the assay of neomycin under *Antibiotics—Microbial Assays* (81), the *Test Dilution* being prepared as follows. Blend an accurately counted number of Boluses (not less than 2) at high speed in a blender jar with a sufficient accurately measured volume of *Buffer*

B.3. (CN 1-May-2017) to obtain a stock solution having a convenient concentration. Dilute this stock solution quantitatively and stepwise with **Buffer B.3.** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin Sulfate

Neomycin sulfate.
Neomycins sulfate [1405-10-3].

» Neomycin Sulfate is the sulfate salt of a kind of neomycin, an antibacterial substance produced by the growth of *Streptomyces fradiae* Waksman (Fam. Streptomycetaceae), or a mixture of two or more such salts. It has a potency equivalent to not less than 600 µg of neomycin per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.

USP Reference standards (11)—

USP Endotoxin RS
USP Neomycin Sulfate RS

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: Dissolve about 10 mg in 1 mL of water, add 5 mL of 15 N sulfuric acid, and heat at 100° for 100 minutes. Allow to cool, add 10 mL of xylene, and shake for 10 minutes. Allow to separate, and decant the xylene layer. To the xylene layer add 10 mL of *p*-bromoaniline TS, and shake: a vivid pink-red color develops upon standing.

C: A solution (1 in 20) responds to the tests for *Sulfate* (191).

pH (791): between 5.0 and 7.5, in a solution containing 33 mg of neomycin per mL.

Loss on drying (731)—Dry about 100 mg in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 8.0% of its weight.

Other requirements—Where the label states that Neomycin Sulfate is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Neomycin for Injection*. Where the label states that Neomycin Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Neomycin for Injection*. Where it is intended for use in preparing nonparenteral sterile dosage forms, it is exempt from the requirements for *Bacterial endotoxins*.

Assay—Proceed with Neomycin Sulfate as directed under *Antibiotics—Microbial Assays* (81).

Neomycin Sulfate Cream

» Neomycin Sulfate Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.

USP Reference standards (11)—

USP Neomycin Sulfate RS

Thin-layer chromatographic identification test

(201BNP): meets the requirements.

Minimum fill (755): meets the requirements.

Change to read:

Assay—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Cream, equivalent to about 1.75 mg of neomycin, shaken in a separator with about 50 mL of ether, and extracted with four 20-mL portions of **Buffer B.3.** (CN 1-May-2017). Combine the aqueous extracts, and dilute with **Buffer B.3.** (CN 1-May-2017) to an appropriate volume to obtain a stock solution of convenient concentration. Dilute this stock solution quantitatively and stepwise with **Buffer B.3.** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin Sulfate Ointment

» Neomycin Sulfate Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.

USP Reference standards (11)—

USP Neomycin Sulfate RS

Thin-layer chromatographic identification test

(201BNP): meets the requirements.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Change to read:

Assay—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Ointment, equivalent to about 3.5 mg of neomycin, shaken in a separator with about 50 mL of ether, and extracted with four 20-mL portions of **Buffer B.3.** (CN 1-May-2017). Combine the aqueous extracts, and dilute with **Buffer B.3.** (CN 1-May-2017) to an appropriate volume to obtain a stock solution of convenient concentration. Dilute this stock solution quantitatively and stepwise with **Buffer B.3.** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin Sulfate Ophthalmic Ointment

DEFINITION

Neomycin Sulfate Ophthalmic Ointment is a sterile preparation of Neomycin Sulfate in a suitable ointment base. It contains the equivalent of NLT 90.0% and NMT 135.0% of the labeled amount of neomycin.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201BNP): Meets the requirements

ASSAY• **PROCEDURE**

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of the Ophthalmic Ointment containing nominally 3.5 mg of neomycin in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–135.0%

SPECIFIC TESTS

- **STERILITY TESTS** (71): Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** (11)
USP Neomycin Sulfate RS

Neomycin Sulfate Oral Solution

» Neomycin Sulfate Oral Solution contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of neomycin. It may contain one or more suitable colors, flavors, and preservatives.

Packaging and storage—Preserve in tight, light-resistant containers, preferably at controlled room temperature.

USP Reference standards (11)—
USP Neomycin Sulfate RS

Thin-layer chromatographic identification test (201BNP): meets the requirements.

Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

pH (791): between 5.0 and 7.5.

Change to read:

Assay—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Oral Solution quantitatively diluted with *Buffer B.3* (CN 1-May-2017) to yield a solution having a convenient concentration of neomycin. Quantitatively dilute this stock solution with *Buffer B.3* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin Sulfate Tablets

» Neomycin Sulfate Tablets contain the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of neomycin.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—
USP Neomycin Sulfate RS

Thin-layer chromatographic identification test (201BNP)—

*Test solution—*Shake a portion of ground Tablet powder, equivalent to about 70 mg of neomycin (base), with 5 mL of water, and filter. Dilute a portion of this solution with 0.1 N hydrochloric acid to obtain a solution containing the equivalent of 3.5 mg of neomycin (base) per mL. It meets the requirements.

Disintegration (701): 60 minutes.

Uniformity of dosage units (905): meet the requirements.

Loss on drying (731)—Dry about 100 mg of powdered Tablets, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 10.0% of its weight.

Change to read:

Assay—Proceed as directed under *Antibiotics—Microbial Assays* (81), using not less than 5 Tablets blended at high-speed in a blender jar for 3 to 5 minutes with a sufficient accurately measured volume of *Buffer B.3* (CN 1-May-2017) to obtain a stock solution having a convenient concentration. Dilute this stock solution quantitatively and stepwise with *Buffer B.3* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin Sulfate and Bacitracin Ointment

» Neomycin Sulfate and Bacitracin Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and bacitracin.

Packaging and storage—Preserve in tight, light-resistant containers, preferably at controlled room temperature.

USP Reference standards (11)—
USP Bacitracin Zinc RS
USP Neomycin Sulfate RS

Thin-layer chromatographic identification test (201BNP): meets the requirements.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for neomycin—Proceed with Ointment as directed in the Assay under *Neomycin Sulfate Ointment*.

Assay for bacitracin—Proceed with Ointment as directed in the Assay under *Bacitracin Ointment*.

Neomycin Sulfate and Bacitracin Zinc Ointment

» Neomycin Sulfate and Bacitracin Zinc Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and bacitracin.

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.

USP Reference standards (11)—

USP Bacitracin Zinc RS

USP Neomycin Sulfate RS

Thin-layer chromatographic identification test (201BNP): meets the requirements.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for neomycin and Assay for bacitracin—Proceed with Ointment as directed in the Assay for neomycin and in the Assay for bacitracin under Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.

Neomycin Sulfate and Dexamethasone Sodium Phosphate Cream

DEFINITION

Neomycin Sulfate and Dexamethasone Sodium Phosphate Cream contains the equivalent of NLT 90.0% and NMT 135.0% of the labeled amount of neomycin, and the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201BNP): Meets the requirements for neomycin
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay for Dexamethasone Phosphate.

ASSAY

• NEOMYCIN

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Cream, containing nominally 1.75 mg of neomycin, with 50 mL of ether, and extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* with a neomycin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–135.0%

• DEXAMETHASONE PHOSPHATE

Solution A: 6.9 g/L of monobasic sodium phosphate

Mobile phase: Methanol and *Solution A* (52:48)

Diluent: Dissolve 0.29 g of dibasic sodium phosphate in 450 mL of water, and add 550 mL of alcohol.

Standard solution: 33 µg/mL of USP Dexamethasone Sodium Phosphate RS in *Diluent*. Prepare this solution freshly.

Sample solution: Nominally 30 µg/mL of dexamethasone phosphate from Cream in *Diluent*, prepared as follows. Transfer a portion of Cream, containing nominally 3 mg of dexamethasone phosphate, to a suitable beaker. Add 65 mL of *Diluent*, and heat just to

boiling. Pour the contents of the beaker into a separatory funnel containing 45 mL of isooctane. After shaking for 1 min, decant the lower layer into a 100-mL volumetric flask. Rinse the beaker with two 15-mL portions of *Diluent*, add each to the separatory funnel, shake, and decant the lower layer from each extraction into the 100-mL volumetric flask. Dilute with *Diluent* to volume, and mix. Pass through a suitable filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for dexamethasone phosphate is about 8.5 min.]

Suitability requirements

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Dexamethasone Sodium Phosphate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of dexamethasone phosphate in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of dexamethasone phosphate, 472.44

M_{r2} = molecular weight of dexamethasone sodium phosphate, 516.40

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **MINIMUM FILL** (755): Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers.
- **USP REFERENCE STANDARDS** (11)
USP Dexamethasone Sodium Phosphate RS
USP Neomycin Sulfate RS

Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Ointment

DEFINITION

Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Ointment is a sterile ointment containing Neomycin Sulfate and Dexamethasone Sodium Phosphate. It contains the equivalent of NLT 90.0% and NMT 135.0% of the labeled amount of neomycin, and the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$).

[NOTE—Where Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Ointment is prescribed without reference to the quantity of neomycin or dexamethasone phosphate contained therein, a product containing 3.5 mg of neomycin and 0.5 mg of dexamethasone phosphate per g shall be dispensed.]

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201BNP):** Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Dexamethasone Phosphate*.

ASSAY• **NEOMYCIN**

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a weighed portion of Ophthalmic Ointment in a separator with about 50 mL of ether, and extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–135.0%

• **DEXAMETHASONE PHOSPHATE**

Buffer: 6.9 g/L of monobasic sodium phosphate

Mobile phase: Methanol and *Buffer* (52:48)

Diluent: Dissolve 0.29 g of dibasic sodium phosphate in 450 mL of water, and add 550 mL of alcohol.

Standard solution: 33 µg/mL of USP Dexamethasone Sodium Phosphate RS in *Diluent*. Prepare this solution freshly.

Sample solution: Nominally 30 µg/mL of dexamethasone phosphate, prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 3 mg of dexamethasone phosphate to a suitable beaker. Add 65 mL of *Diluent*, and heat just to boiling. Pour the contents of the beaker into a separator containing 45 mL of isooctane. After shaking for 1 min, decant the lower layer into a 100-mL volumetric flask. Rinse the beaker with two 15-mL portions of *Diluent*, extracting the remaining isooctane in the separator with each portion, and decanting the lower layer from each extraction into the 100-mL volumetric flask. Dilute with *Diluent* to volume, and mix. Pass through a suitable filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for dexamethasone phosphate is about 8.5 min.]

Suitability requirements

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Dexamethasone Sodium Phosphate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of dexamethasone phosphate in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of dexamethasone phosphate, 472.44

M_{r2} = molecular weight of dexamethasone sodium phosphate, 516.40

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **STERILITY TESTS (71):** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS (11)**
USP Dexamethasone Sodium Phosphate RS
USP Neomycin Sulfate RS

Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Solution

DEFINITION

Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Solution is a sterile, aqueous solution of Neomycin Sulfate and Dexamethasone Sodium Phosphate. It contains the equivalent of NLT 90.0% and NMT 130.0% of the labeled amount of neomycin, and the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$). It may contain one or more suitable buffers, dispersants, and preservatives.

[NOTE—Where Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Solution is prescribed, without reference to the amount of neomycin or dexamethasone phosphate contained therein, a product containing 3.5 mg/mL of neomycin and 1.0 mg/mL of dexamethasone phosphate shall be dispensed.]

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201BNP):** Meets the requirements for neomycin
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Dexamethasone Phosphate*.

ASSAY• **NEOMYCIN**

(See *Antibiotics—Microbial Assays* (81).)

Analysis: Dilute an aliquot of Ophthalmic Solution with *Buffer B.3* to obtain a *Test Dilution* with a neomycin concentration that is nominally equivalent to the median level of the standard (1.0 µg/mL).

Acceptance criteria: 90.0%–130.0%

• **DEXAMETHASONE PHOSPHATE**

Solution A: 0.29 g/L of dibasic sodium phosphate

Solution B: 13.80 g/L of monobasic sodium phosphate

Mobile phase: Acetonitrile and *Solution B* (31:69)

Standard solution: 27 µg/mL of USP Dexamethasone Sodium Phosphate RS in *Solution A*. Pass through a filter of 1-µm or finer pore size.

Sample solution: Nominally 25 µg/mL of dexamethasone phosphate from Ophthalmic Solution in *Solution A* prepared as follows. Slowly dilute a portion of Ophthalmic Solution with *Solution A* to volume, mix, and pass through a suitable filter of 1-µm or finer pore size.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 3.9-mm × 30-cm; 10-μm packing L1**Flow rate:** 1.3 mL/min**Injection volume:** 50 μL**System suitability****Sample:** *Standard solution*

[NOTE—The retention time for dexamethasone phosphate is about 8.5 min.]

Suitability requirements**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) in each mL of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak area from the *Sample solution*
 r_S = peak area from the *Standard solution*
 C_S = concentration of USP Dexamethasone Sodium Phosphate RS in the *Standard solution* (μg/mL)
 C_U = nominal concentration of dexamethasone phosphate in the *Sample solution* (μg/mL)
 M_{r1} = molecular weight of dexamethasone phosphate, 472.44
 M_{r2} = molecular weight of dexamethasone sodium phosphate, 516.40
Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

- **STERILITY TESTS** (71): Meets the requirements
- **PH** (791): 6.0–8.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** (11)
USP Dexamethasone Sodium Phosphate RS
USP Neomycin Sulfate RS

Neomycin Sulfate and Fluocinolone Acetonide Cream

» Neomycin Sulfate and Fluocinolone Acetonide Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluocinolone acetonide ($C_{24}H_{30}F_2O_6$).

Packaging and storage—Preserve in collapsible tubes or in tight containers.

USP Reference standards (11)—USP Fluocinolone Acetonide RS
USP Neomycin Sulfate RS**Identification**—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: It meets the requirements for the *Identification test* under *Fluocinolone Acetonide Cream*.

Minimum fill (755): meets the requirements.

Assay for neomycin—Proceed with Cream as directed in the *Assay* under *Neomycin Sulfate Cream*.

Assay for fluocinolone acetonide—Proceed with Cream as directed in the *Assay* under *Fluocinolone Acetonide Cream*.

Neomycin Sulfate and Fluorometholone Ointment

» Neomycin Sulfate and Fluorometholone Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluorometholone ($C_{22}H_{29}FO_4$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.

USP Reference standards (11)—

USP Fluorometholone RS

USP Neomycin Sulfate RS

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: The ratios of the retention time of the main peak to that of the internal standard peak obtained from the *Standard preparation* and the *Assay preparation* as directed in the *Assay for fluorometholone* do not differ by more than 2.0%.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for neomycin—Proceed with Ointment as directed in the *Assay* under *Neomycin Sulfate Ointment*.

Assay for fluorometholone—

Internal standard solution, Mobile solvent, and Standard preparation—Prepare as directed in the *Assay* under *Fluorometholone Cream*.

Assay preparation—Transfer an accurately weighed quantity of Ointment, equivalent to about 1 mg of fluorometholone, to a suitable container, add 20.0 mL of *Internal standard solution*, and mix.

Procedure—Treat 20.0 mL each of the *Standard preparation* and the *Assay preparation* in the following manner. To each add 10.0 mL of hexane, shake for about 15 minutes, then allow the layers to separate, and centrifuge, if necessary. Using the lower (acetonitrile) layer, proceed as directed for *Procedure* in the *Assay* under *Fluorometholone Cream*, beginning with "Using a suitable microsyringe." Calculate the quantity, in mg, of $C_{22}H_{29}FO_4$ in the portion of Ointment taken by the formula:

$$20C(R_U / R_S)$$

in which the terms are as defined therein.

Neomycin Sulfate and Flurandrenolide Cream

» Neomycin Sulfate and Flurandrenolide Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of

the labeled amount of flurandrenolide ($C_{24}H_{33}FO_6$).

Packaging and storage—Preserve in collapsible tubes or in tight containers, protected from light.

USP Reference standards (11)—

USP Flurandrenolide RS

USP Neomycin Sulfate RS

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: It meets the requirements for the *Identification test* under *Flurandrenolide Cream*.

Minimum fill (755): meets the requirements.

Assay for neomycin—Proceed with Cream as directed in the *Assay* under *Neomycin Sulfate Ointment*.

Assay for flurandrenolide—Proceed with Cream as directed in the *Assay* under *Flurandrenolide Cream*. Calculate the quantity, in mg, of $C_{24}H_{33}FO_6$ in the portion of Cream taken by the formula:

$$10C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate and Flurandrenolide Lotion

» Neomycin Sulfate and Flurandrenolide Lotion contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide ($C_{24}H_{33}FO_6$).

Packaging and storage—Preserve in tight containers, protected from light.

USP Reference standards (11)—

USP Flurandrenolide RS

USP Neomycin Sulfate RS

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: It meets the requirements for the *Identification test* under *Flurandrenolide Cream*.

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Minimum fill (755): meets the requirements.

Change to read:

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Lotion, equivalent to about 3.5 mg of neomycin, blended for 3 to 5 minutes in a high-speed glass blender jar containing an accurately measured volume of *Buffer B.3* (CN 1-May-2017) sufficient to obtain a stock solution having a convenient concentration of neomycin. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.3* (CN 1-May-2017) to obtain a *Test Dilution*

having a concentration of neomycin assumed to be equal to the median dose level of the *Standard*.

Assay for flurandrenolide—Proceed with Neomycin Sulfate and Flurandrenolide Lotion as directed in the *Assay* under *Flurandrenolide Cream*. Calculate the quantity, in mg, of $C_{24}H_{33}FO_6$ in the portion of Lotion taken by the formula:

$$10C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate and Flurandrenolide Ointment

» Neomycin Sulfate and Flurandrenolide Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide ($C_{24}H_{33}FO_6$).

Packaging and storage—Preserve in collapsible tubes or in tight containers, protected from light.

USP Reference standards (11)—

USP Flurandrenolide RS

USP Neomycin Sulfate RS

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: It meets the requirements for the *Identification test* under *Flurandrenolide Cream*.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for neomycin—Proceed with Ointment as directed in the *Assay* under *Neomycin Sulfate Ointment*.

Assay for flurandrenolide—Proceed with Ointment as directed in the *Assay* under *Flurandrenolide Cream*. Calculate the quantity, in mg, of $C_{24}H_{33}FO_6$ in the portion of Ointment taken by the formula:

$$10C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate and Gramicidin Ointment

» Neomycin Sulfate and Gramicidin Ointment contains the equivalent of not less than 90.0 percent and not more than 140.0 percent of the labeled amounts of neomycin and gramicidin.

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.

USP Reference standards (11)—

USP Gramicidin RS

USP Neomycin Sulfate RS

Thin-Layer Chromatographic Identification Test

(201BNP): meets the requirements.

Minimum fill (755): meets the requirements.**Water Determination, Method I** (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.**Assay for neomycin**—Proceed with Ointment as directed in the Assay under *Neomycin Sulfate Ointment*.**Assay for gramicidin**—Proceed as directed for gramicidin under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Ointment dissolved in 50 mL of hexanes in a separator, and extracted with four 20-mL portions of 80 percent alcohol. Combine the extracts in a suitable volumetric flask, dilute with alcohol to volume, and mix. Dilute this solution quantitatively and stepwise with alcohol to obtain a *Test Dilution* having a concentration of gramicidin assumed to be equal to the median dose level of the Standard.**Neomycin Sulfate and Hydrocortisone Cream**

» Neomycin Sulfate and Hydrocortisone Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.**USP Reference standards** (11)—

USP Hydrocortisone RS

USP Neomycin Sulfate RS

Identification—A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* (201BNP).B: The retention time of the major peaks for hydrocortisone in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for hydrocortisone*.**Minimum fill** (755): meets the requirements.**Assay for neomycin**—Proceed with Cream as directed in the Assay under *Neomycin Sulfate Ointment*.**Assay for hydrocortisone**—Proceed with Cream as directed in the Assay for hydrocortisone under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment*.**Neomycin Sulfate and Hydrocortisone Ointment**

» Neomycin Sulfate and Hydrocortisone Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.**USP Reference standards** (11)—

USP Hydrocortisone RS

USP Neomycin Sulfate RS

Identification—A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* (201BNP).B: The retention time of the major peak for hydrocortisone in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for hydrocortisone*.**Minimum fill** (755): meets the requirements.**Water Determination, Method I** (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.**Assay for neomycin**—Proceed with Ointment as directed in the Assay under *Neomycin Sulfate Ointment*.**Assay for hydrocortisone**—Proceed with Ointment as directed in the Assay for hydrocortisone under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment*.**Neomycin Sulfate and Hydrocortisone Otic Suspension**

» Neomycin Sulfate and Hydrocortisone Otic Suspension is a sterile suspension containing not less than 90.0 percent and not more than 130.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone. It contains Acetic Acid, and may contain one or more suitable buffers, dispersants, and preservatives.

NOTE—Where Neomycin Sulfate and Hydrocortisone Otic Suspension is prescribed, without reference to the quantity of neomycin or hydrocortisone contained therein, a product containing 3.5 mg of neomycin and 10 mg of hydrocortisone per mL shall be dispensed.

Packaging and storage—Preserve in tight, light-resistant containers.**USP Reference standards** (11)—

USP Hydrocortisone RS

USP Neomycin Sulfate RS

Sterility Tests (71): meets the requirements.**pH** (791): between 4.5 and 6.0.**Assay for neomycin**—Using an accurately measured volume of Otic Suspension, freshly mixed and free from entrapped air, proceed as directed in the Assay for neomycin under *Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution*.**Assay for hydrocortisone**—

Mobile phase and Standard preparation—Prepare as directed in the Assay for hydrocortisone content under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment*.

Assay preparation—Transfer 3.0 mL of Otic Suspension, freshly mixed and free from entrapped air, to a 200-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. Filter the solution, rejecting the first 10 mL of the filtrate.

Procedure—Proceed as directed for *Procedure* in the *Assay for hydrocortisone content under Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment*. Calculate the quantity, in mg, of $C_{21}H_{30}O_5$ in each mL of the Otic Suspension taken by the formula:

$$(66.67C)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate and Hydrocortisone Acetate Cream

» Neomycin Sulfate and Hydrocortisone Acetate Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Hydrocortisone Acetate RS

USP Neomycin Sulfate RS

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: The retention time of the major peak for hydrocortisone acetate in the chromatogram of the *Assay preparation*, corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for hydrocortisone acetate*.

Minimum fill (755): meets the requirements.

Assay for neomycin—Proceed with Cream as directed in the *Assay under Neomycin Sulfate Ointment*.

Assay for hydrocortisone acetate—Proceed with Cream as directed in the *Assay under Hydrocortisone Acetate Lotion*.

Neomycin Sulfate and Hydrocortisone Acetate Lotion

» Neomycin Sulfate and Hydrocortisone Acetate Lotion contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Hydrocortisone Acetate RS

USP Neomycin Sulfate RS

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: The retention time of the major peak for hydrocortisone acetate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for hydrocortisone acetate*.

Minimum fill (755): meets the requirements.

Change to read:

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* (81), blending an accurately measured volume of Lotion for 3 to 5 minutes in a high-speed glass blender jar containing an accurately measured volume of *Buffer B.3* (CN 1-May-2017). Dilute an accurately measured volume of the solution so obtained quantitatively and stepwise with *Buffer B.3* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of neomycin assumed to be equal to the median dose level of the *Standard*.

Assay for hydrocortisone acetate—Proceed with Lotion as directed in the *Assay under Hydrocortisone Acetate Lotion*.

Neomycin Sulfate and Hydrocortisone Acetate Ointment

» Neomycin Sulfate and Hydrocortisone Acetate Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.

USP Reference standards (11)—

USP Hydrocortisone Acetate RS

USP Neomycin Sulfate RS

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: The retention time of the major peak for hydrocortisone acetate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for hydrocortisone acetate*.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for neomycin—Proceed with the Ointment as directed in the *Assay under Neomycin Sulfate Ointment*.

Assay for hydrocortisone acetate—Proceed with the Ointment as directed in the *Assay under Hydrocortisone Acetate Lotion*.

Neomycin Sulfate and Hydrocortisone Acetate Ophthalmic Suspension

» Neomycin Sulfate and Hydrocortisone Acetate Ophthalmic Suspension is a sterile, aqueous suspension containing the equivalent of not less than 90.0 percent and not more than 130.0 per-

cent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards (11)—

USP Hydrocortisone Acetate RS

USP Neomycin Sulfate RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay for hydrocortisone acetate* exhibits a major peak for hydrocortisone acetate, the retention time of which corresponds with that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay for hydrocortisone acetate*.

Sterility Tests (71): meets the requirements.

pH (791): between 5.5 and 7.5.

Change to read:

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.3* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for hydrocortisone acetate—

Mobile phase—Prepare a solution containing *n*-butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6).

Internal standard solution—Prepare a solution of fluoxymesterone in chloroform containing 0.8 mg per mL.

Standard preparation—Dissolve about 10 mg of USP Hydrocortisone Acetate RS, accurately weighed, in 10.0 mL of *Internal standard solution*, dilute with about 40 mL of chloroform, and mix.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, equivalent to about 10 mg of hydrocortisone acetate, to a suitable container. Add 10.0 mL of *Internal standard solution* and about 40 mL of chloroform, shake vigorously for about 5 minutes, and allow the phases to separate. Use the clear chloroform layer as the *Assay preparation*.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm \times 30-cm column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the analyte and internal standard peaks is not less than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 15 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 and 1.0 for hydrocortisone acetate and fluoxymesterone, respectively. Calculate the quantity, in mg, of hydrocortisone acetate ($C_{23}H_{32}O_6$) in each mL of the Ophthalmic Suspension taken by the formula:

$$(W/V)(R_U/R_S)$$

in which *W* is the quantity, in mg, of USP Hydrocortisone Acetate RS taken to prepare the *Standard preparation*, *V* is the volume, in mL, of Ophthalmic Suspension taken, and *R_U* and *R_S* are the peak response ratios of the hydrocortisone acetate peak to the internal standard peak obtained from

the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate, Isoflupredone Acetate, and Tetracaine Hydrochloride Ointment

» Neomycin Sulfate, Isoflupredone Acetate, and Tetracaine Hydrochloride Ointment contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of neomycin, and not less than 92.5 percent and not more than 117.5 percent of the labeled amounts of isoflupredone acetate ($C_{23}H_{29}FO_6$) and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in a suitable ointment base.

Packaging and storage—Preserve in collapsible tubes or well-closed containers.

Labeling—Label it to indicate that it is intended for veterinary use only.

USP Reference standards (11)—

USP Isoflupredone Acetate RS

USP Neomycin Sulfate RS

USP Tetracaine Hydrochloride RS

Identification—

A: Thin-Layer Chromatographic Identification Test (201)—

Test solution—To 2 g of Ointment in a centrifuge tube add 25 mL of chloroform, and heat at 60° for 5 minutes, with occasional shaking. Centrifuge, discard the chloroform layer, add 5 mL of water, shake, and filter. Use the filtrate.

Standard solution—Prepare a solution containing 2 mg of USP Neomycin Sulfate RS per mL.

Application volume: 1 μ L.

Developing solvent system: a mixture of water, butyl alcohol, glacial acetic acid, and pyridine (35:30:22:6).

Spray reagent: a solution of triketohydrindene hydrate in butyl alcohol (2 in 1000).

Procedure—Proceed as directed in the chapter, except to locate the spots by spraying with *Spray reagent* and heating at 100° for 5 minutes.

B: The retention time of the major peak for isoflupredone acetate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for isoflupredone acetate*.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 1.0%, a mixture of methanol and chloroform (3:2) being used instead of methanol in the titration vessel and the titration vessel being heated to between 45° and 55°.

Change to read:

Assay for neomycin—Proceed as directed under *Antibiotics—Microbial Assays* (81). Place an accurately weighed portion of Ointment in a centrifuge tube with 25 mL of chloroform. Heat at 60° for 3 minutes, shake until the Ointment is dissolved, centrifuge, and remove and discard the chloroform. Add 15 mL of chloroform, shake, centrifuge, and remove and discard the chloroform. Add 5.0 mL of water and 15 mL of chromatographic *n*-heptane, shake, centrifuge, and remove and discard the *n*-heptane layer. Dilute an accurately measured volume of the water layer quantitatively with *Buffer B.3* (CN 1-May-2017) to obtain a *Test Dilution* having

a concentration of neomycin assumed to be equal to the median dose level of the Standard.

Assay for isoflupredone acetate—

Mobile phase, Diluent, Internal standard solution, Standard preparation, and Chromatographic system—Proceed as directed in the Assay under Isoflupredone Acetate.

Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 4 mg of isoflupredone acetate, to a suitable container. Add 8.0 mL of *Internal standard solution*, 32.0 mL of *Diluent*, and about 10 glass beads. Shake for about 15 minutes, centrifuge, and use the clear chloroform portion.

Procedure—Proceed as directed in the Assay under Isoflupredone Acetate. Calculate the quantity, in mg, of isoflupredone acetate ($C_{23}H_{29}FO_6$) in the portion of Ointment taken by the formula:

$$W_S(R_U / R_S)$$

in which the terms are as defined therein.

Assay for tetracaine hydrochloride—

Standard preparation—Prepare a solution in chloroform having a known concentration of about 5.0 µg of USP Tetracaine Hydrochloride RS per mL.

Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 1.25 mg of tetracaine hydrochloride, to a 250-mL volumetric flask, add about 100 mL of chloroform, and warm on a steam bath for about 3 minutes to dissolve the Ointment. Cool to room temperature, dilute with chloroform to volume, and mix.

Blank solution—Transfer an accurately weighed portion of the ointment base, equivalent to the weight used in the *Assay preparation*, to a 250-mL volumetric flask. Add 100 mL of chloroform, warm on a steam bath for about 3 minutes to dissolve, and allow to stand until the solution has equilibrated to room temperature. Dilute with chloroform to volume, and mix well.

Procedure—Concomitantly determine the absorbances of the *Standard preparation*, the *Blank solution*, and the *Assay preparation* with a suitable spectrophotometer at the wavelength of maximum absorbance at about 310 nm, using chloroform to zero the instrument. Calculate the absorbance of the *Blank solution*, A_B , adjusted for weight difference between the *Assay preparation* and the *Blank solution*, by the formula:

$$A(W_T / W_B)$$

in which A is the absorbance of the *Blank solution*; W_T is the weight, in mg, of Ointment taken to prepare the *Assay preparation*; and W_B is the weight, in mg, of the ointment base taken to prepare the *Blank solution*. Calculate the quantity, in mg, of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Ointment taken by the formula:

$$250C [(A_U - A_B) / A_S]$$

in which C is the concentration, in mg per mL, of USP Tetracaine Hydrochloride RS in the *Standard preparation*; A_B is as obtained above; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate, Isoflupredone Acetate, and Tetracaine Hydrochloride Topical Powder

» Neomycin Sulfate, Isoflupredone Acetate, and Tetracaine Hydrochloride Topical Powder contains

the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 120.0 percent of the labeled amounts of isoflupredone acetate ($C_{23}H_{29}FO_6$) and tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$).

Packaging and storage—Preserve in well-closed containers.

Labeling—Label it to indicate that it is intended for veterinary use only.

USP Reference standards (11)—

USP Isoflupredone Acetate RS

USP Neomycin Sulfate RS

USP Tetracaine Hydrochloride RS

Identification—

A: Thin-Layer Chromatographic Identification Test (201)—

Test solution—Add 20 mL of chloroform to 1 g of Topical Powder, shake for 5 to 10 minutes, and centrifuge. Evaporate a 10-mL portion of the clear solution to dryness, and dissolve the residue in 1 mL of a mixture of chloroform and alcohol (1:1).

Standard solution: 0.5 mg of USP Isoflupredone Acetate RS per mL, in a mixture of chloroform and alcohol (1:1).

Application volume: 30 µL.

Developing solvent system: a mixture of methylene chloride and methanol (180:16), in a paper-lined chromatographic chamber.

Spray reagent: a solution of sulfuric acid in methanol (70 in 100).

Procedure—Proceed as directed in the chapter, except to use a plate that has been activated by heating in an oven at 105° for 60 minutes. Allow the plate to cool before using. Locate the spots under short- and long-wavelength UV light. Spray the plate with *Spray reagent*, heat at 90° for 30 minutes, and locate the spots under short- and long-wavelength UV light (presence of isoflupredone acetate).

B: Thin-Layer Chromatographic Identification Test (201)—

Test solution—To 1 g of Topical Powder in a centrifuge tube add 5 mL of water, and shake until dissolved. Prepare a suspension of 10 g of cation-exchange resin in 10 mL of water, add 5 mL of a solution of sodium hydroxide (1 in 2), mix, and wash the resin with water until the pH of the wash is about 9. Add 0.3 g of this suspension to the solution of Topical Powder, and shake for 10 seconds. Centrifuge for 1 minute, and discard the supernatant. Wash the resin in the tube with 10 mL of water, centrifuge, and discard the supernatant. Add 2 mL of 1 M ammonium hydroxide, shake for 10 seconds, and filter. Use the filtrate.

Standard solution, Application volume, Developing solvent system, Spray reagent, and Procedure—Proceed as directed in Identification test A under Neomycin Sulfate, Isoflupredone Acetate, and Tetracaine Hydrochloride Ointment (presence of neomycin).

Minimum fill (755): meets the requirements.

Loss on drying (731)—Dry about 2 g in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 8.0% of its weight.

Change to read:

Assay for neomycin—Proceed as directed under Antibiotics—Microbial Assays (81), the *Test Dilution* being prepared as follows. Use an accurately weighed quantity of Topical Powder diluted quantitatively with *Buffer B.3* (CN 1-May-2017) to obtain a solution having a suitable concentration of neomycin. Dilute this stock solution quantitatively with *Buffer*

B.3. (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for isoflupredone acetate—

Mobile phase, Diluent, Internal standard solution, Standard preparation, and Chromatographic system—Proceed as directed in the Assay under Isoflupredone Acetate.

Assay preparation—Transfer an accurately weighed portion of Topical Powder, equivalent to about 4 mg of isoflupredone acetate, to a suitable container. Add 8.0 mL of *Internal standard solution*, 32.0 mL of *Diluent*, and about 10 glass beads. Shake for about 15 minutes, centrifuge, and use the clear chloroform portion.

Procedure—Proceed as directed in the Assay under Isoflupredone Acetate. Calculate the quantity, in mg, of isoflupredone acetate ($C_{23}H_{29}FO_6$) in the portion of Topical Powder taken by the formula:

$$W_S(R_U / R_S)$$

in which the terms are as defined therein.

Assay for tetracaine hydrochloride—

Standard preparation—Prepare a solution having a known concentration of 5.5 µg of USP Tetracaine Hydrochloride RS per mL.

Assay preparation—Transfer an accurately weighed portion of Topical Powder, equivalent to about 5.5 mg of tetracaine hydrochloride, to a 100-mL volumetric flask, dilute with water to volume, and mix. Pass about 30 mL through a fine, sintered-glass filter. Transfer 10.0 mL of the clear filtrate to a second 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the wavelength of maximum absorbance at about 310 nm, with a suitable spectrophotometer, using water to zero the instrument. Calculate the quantity, in mg, of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$) in the portion of Topical Powder taken by the formula:

$$1000C(A_U / A_S)$$

in which *C* is the concentration, in mg per mL, of USP Tetracaine Hydrochloride RS in the *Standard preparation*; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate and Methylprednisolone Acetate Cream

DEFINITION

Neomycin Sulfate and Methylprednisolone Acetate Cream contains the equivalent of NLT 90.0% and NMT 135.0% of the labeled amount of neomycin, and NLT 90.0% and NMT 110.0% of the labeled amount of methylprednisolone acetate ($C_{24}H_{32}O_6$).

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201BNP): Meets the requirements for neomycin

• B.

Solution A, Solution B, Standard solution, Sample solution, Adsorbent, Application volume, and Developing solvent system: Proceed as directed in the Assay for Methylprednisolone Acetate.

Analysis: Proceed with thin-layer chromatography as directed in the Assay for Methylprednisolone Acetate.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• NEOMYCIN

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Cream containing nominally 3.5 mg of neomycin in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–135.0%

• METHYLPREDNISOLONE ACETATE

Solution A: Alcohol and chloroform (1:1)

Solution B: Alcohol and tetramethylammonium hydroxide TS (9:1)

Standard solution: 500 µg/mL of USP Methylprednisolone Acetate RS in *Solution A*

Sample solution: Transfer a portion of Cream containing nominally 5 mg of methylprednisolone acetate to a 125-mL separator, and add 50 mL of solvent hexane. Extract with three 10-mL portions of acetonitrile, and evaporate the combined extracts on a steam bath with the aid of a current of air nearly to dryness. Transfer the residue to a 10-mL volumetric flask with the aid of one 5-mL portion and two 2-mL portions of *Solution A*. Dilute with *Solution A* to volume.

Adsorbent: 0.5-mm layer of chromatographic silica gel mixture (see *Chromatography* (621))

Application volume: 250 µL

Developing solvent system: Ethyl acetate and chloroform (7:5)

Analysis

Samples: *Standard solution* and *Sample solution*

Divide the plate into three equal sections, the left and right sections to be used for the *Sample solution* and *Standard solution*, respectively, and the center section for the blank. Apply the solutions as streaks 2.5 cm from the bottom of the designated section of the plate, and dry the streaks with the aid of a current of air. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Locate the principal bands from the *Standard solution* and the *Sample solution* (see also *Identification test B*) by viewing under short-wavelength UV light. Mark these bands and the corresponding band in the section of the plate representing the blank. Quantitatively remove the silica gel containing these bands, and transfer to separate glass-stoppered, 50-mL centrifuge tubes. Add 25.0 mL of alcohol to each tube, shake for 2 min, and centrifuge at about 1500 rpm for 5 min. Transfer 20.0 mL of each supernatant to separate glass-stoppered, 50-mL conical flasks. Add 2.0 mL of blue tetrazolium TS to each solution, and to each flask, add 2.0 mL of *Solution B*. Mix, and allow the solutions to stand in the dark for 90 min.

Instrumental conditions

Mode: Vis

Analytical wavelength: Maximum at about 525 nm

Cell: 1 cm

Calculate the percentage of the labeled amount of methylprednisolone acetate ($C_{24}H_{32}O_6$) in the portion of Cream taken:

$$\text{Result} = (A_U / A_S) \times (C_S / C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Methylprednisolone Acetate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of methylprednisolone acetate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers, protected from light.
- **USP REFERENCE STANDARDS (11)**
USP Methylprednisolone Acetate RS
USP Neomycin Sulfate RS

Neomycin and Polymyxin B Sulfates Cream

» Neomycin and Polymyxin B Sulfates Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B. It may contain a suitable local anesthetic.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.

USP Reference standards (11)—

USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Thin-layer chromatographic identification test (201BNP): meets the requirements.

Minimum fill (755): meets the requirements.

Change to read:

Assay for neomycin—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Cream, equivalent to about 1.75 mg of neomycin, shaken in a separator with about 50 mL of ether, and extracted with four 20-mL portions of *Buffer B.3* (CN 1-May-2017). Combine the aqueous extracts, and dilute with *Buffer B.3* (CN 1-May-2017) to an appropriate volume to obtain a stock solution of convenient concentration. Dilute this stock solution quantitatively and stepwise with *Buffer B.3* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for polymyxin B—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Cream shaken with about 50 mL of ether in a separator, and extracted with four 25-mL portions of *Buffer B.6* (CN 1-May-2017). Combine the aqueous extracts, and dilute with *Buffer B.6* (CN 1-May-2017) to an appropriate volume to obtain a stock solution. Dilute this stock solution quantitatively and stepwise with *Buffer B.6* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (10 Polymyxin B Units per mL). Add to each test dilution of the Standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6* (CN 1-May-2017), to obtain the same concentration of neomycin present in the *Test Dilution*.

Neomycin and Polymyxin B Sulfates Solution for Irrigation

» Neomycin and Polymyxin B Sulfates Solution for Irrigation is a sterile, aqueous solution containing the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and of polymyxin B. It may contain a suitable preservative.

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate that it is to be diluted for use in a urinary bladder irrigation and is not intended for injection.

USP Reference standards (11)—

USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Thin-layer chromatographic identification test (201BNP): meets the requirements.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

pH (791): between 4.5 and 6.0.

Assay for neomycin and Assay for polymyxin B—Proceed with Neomycin and Polymyxin B Sulfates Solution for Irrigation as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution*.

Neomycin and Polymyxin B Sulfates Ophthalmic Ointment

DEFINITION

Neomycin and Polymyxin B Sulfates Ophthalmic Ointment is a sterile ointment containing Neomycin Sulfate and Polymyxin B Sulfate. It contains the equivalent of NLT 90.0% and NMT 130.0% of the labeled amounts of neomycin and polymyxin B.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201BNP):** Meets the requirements

ASSAY

• NEOMYCIN

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–130.0%

• POLYMYXIN B

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6*. Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 Polymyxin B Units/mL). Add to each *Test Dilution* of the standard, a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*,

to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.

Acceptance criteria: 90.0%–130.0%

SPECIFIC TESTS

- **STERILITY TESTS** (71): Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** (11)
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates Ophthalmic Solution

» Neomycin and Polymyxin B Sulfates Ophthalmic Solution contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B. It may contain one or more suitable buffers, dispersants, irrigants, and preservatives.

Packaging and storage—Preserve in tight containers, and avoid exposure to excessive heat.

USP Reference standards (11)—

USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Thin-layer chromatographic identification test (201BNP): meets the requirements.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH (791): between 5.0 and 7.0.

Assay for neomycin and Assay for polymyxin B—Proceed with Ophthalmic Solution as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution*.

Neomycin and Polymyxin B Sulfates and Bacitracin Ointment

» Neomycin and Polymyxin B Sulfates and Bacitracin Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin. It may contain a suitable local anesthetic.

Packaging and storage—Preserve in tight, light-resistant containers, preferably at controlled room temperature.

USP Reference standards (11)—

USP Bacitracin Zinc RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Thin-layer chromatographic identification test (201BNP): meets the requirements.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for neomycin and Assay for polymyxin B—Proceed with Ointment as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment*.

Assay for bacitracin—Proceed with Ointment as directed in the *Assay* under *Bacitracin Ointment*.

Neomycin and Polymyxin B Sulfates and Bacitracin Ophthalmic Ointment

DEFINITION

Neomycin and Polymyxin B Sulfates and Bacitracin Ophthalmic Ointment is a sterile ointment containing Neomycin Sulfate, Polymyxin B Sulfate, and Bacitracin. It contains the equivalent of NLT 90.0% and NMT 140.0% of the labeled amounts of neomycin, polymyxin B, and bacitracin.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201BNP): Meets the requirements

ASSAY

• NEOMYCIN

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–140.0%

• POLYMYXIN B

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6*. Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 Polymyxin B Units/mL). Add to each *Test Dilution* of the standard, a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.

Acceptance criteria: 90.0%–140.0%

• BACITRACIN

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (1.0 Bacitracin Unit/mL). If the *Sample solution* has a concentration of less than 100 Bacitracin Units/mL, add hydrochloric acid to each *Test Dilution* of the standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of the sample.

Acceptance criteria: 90.0%–140.0%

SPECIFIC TESTS

- **STERILITY TESTS** (71): Meets the requirements
- **OTHER REQUIREMENTS**: It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** (11)
USP Bacitracin Zinc RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates, Bacitracin, and Hydrocortisone Acetate Ointment

» Neomycin and Polymyxin B Sulfates, Bacitracin, and Hydrocortisone Acetate Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate in a suitable ointment base.

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.

USP Reference standards (11)—

USP Bacitracin Zinc RS
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Identification—

A: It meets the requirements under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: The retention time of the major peak for hydrocortisone acetate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for hydrocortisone acetate*.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for neomycin and Assay for polymyxin B—Proceed with Ointment as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment*.

Assay for bacitracin—Proceed with Ointment as directed in the *Assay under Bacitracin Ointment*.

Assay for hydrocortisone acetate—Proceed with Ointment as directed in the *Assay under Hydrocortisone Acetate Lotion*.

Neomycin and Polymyxin B Sulfates, Bacitracin, and Hydrocortisone Acetate Ophthalmic Ointment

DEFINITION

Neomycin and Polymyxin B Sulfates, Bacitracin, and Hydrocortisone Acetate Ophthalmic Ointment contains the

equivalent of NLT 90.0% and NMT 140.0% of the labeled amounts of neomycin, polymyxin B, and bacitracin, and NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone acetate in a suitable ointment base.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201BNP): Meets the requirements
- **B.** The retention time of the hydrocortisone acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Hydrocortisone Acetate*.

ASSAY

• NEOMYCIN

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–140.0%

• POLYMYXIN B

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6*. Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 polymyxin B units/mL). Add to each *Test Dilution* of the standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.

Acceptance criteria: 90.0%–140.0%

• BACITRACIN

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 20-mL portions of *Buffer B.1*. Combine the buffer extracts, and dilute with *Buffer B.1* to a suitable volume.

Analysis: Proceed as directed in the chapter. Add sufficient 0.01 N hydrochloric acid to a portion of the *Sample solution* so that the amount of hydrochloric acid in the *Test Dilution* is the same as in the median level of the standard. Dilute with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–140.0%

• HYDROCORTISONE ACETATE

Mobile phase: Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30)

Standard solution: 0.10 mg/mL of USP Hydrocortisone Acetate RS in water-saturated chloroform

Sample solution: Nominally 0.10 mg/mL of hydrocortisone acetate from Ophthalmic Ointment prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 2.5 mg of hydrocortisone acetate to a closable container. Add 25.0 mL of water-saturated chloroform and about 10 glass beads. Securely close the container, and shake vigorously for approximately 15 min. Centrifuge, and use the clear, lower chloroform layer.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-μm packing L3

System suitabilitySample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Hydrocortisone Acetate RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of hydrocortisone acetate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **STERILITY TESTS** (71): Meets the requirements
- **OTHER REQUIREMENTS**: It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** (11)
 USP Bacitracin Zinc RS
 USP Hydrocortisone Acetate RS
 USP Neomycin Sulfate RS
 USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment

» Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$).

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.

USP Reference standards (11)—

USP Bacitracin Zinc RS
 USP Lidocaine RS
 USP Neomycin Sulfate RS
 USP Polymyxin B Sulfate RS

Identification—

A: It meets the requirements under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: The retention time of the major peak for lidocaine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for lidocaine*.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for neomycin and Assay for polymyxin B—Proceed with Ointment as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment*.

Assay for bacitracin—Proceed with Ointment as directed in the *Assay under Bacitracin Ointment*.

Assay for lidocaine—

Mobile phase—Dissolve 4.44 g of docusate sodium in 1000 mL of a mixture of methanol and water (4:1), add 1 mL of 0.1 N sulfuric acid, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve a suitable quantity of USP Lidocaine RS, accurately weighed, in *Mobile phase* to obtain a solution having a known concentration of about 0.4 mg per mL.

Assay preparation—Transfer an accurately weighed quantity of Ointment, equivalent to about 40 mg of lidocaine, to a separator, add 50 mL of *n*-hexane, and shake until the specimen is in solution. Add 30 mL of *Mobile phase*, shake for 1 minute, and allow the layers to separate. Drain the lower layer into a 100-mL volumetric flask, and extract the *n*-hexane layer remaining in the separator with two 30-mL portions of *Mobile phase*, combining the lower layers in the volumetric flask. Dilute the combined extracts in the 100-mL volumetric flask with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak response as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 500 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of lidocaine ($C_{14}H_{22}N_2O$) in the portion of Ointment taken by the formula:

$$100C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Lidocaine RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ointment

» Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin. It may contain a suitable local anesthetic.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.

USP Reference standards (11)—

USP Bacitracin Zinc RS

USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Thin-layer chromatographic identification test (201BNP): meets the requirements.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for neomycin, Assay for polymyxin B, and Assay for bacitracin—Proceed with Ointment as directed in the Assay for neomycin, in the Assay for polymyxin B, and in the Assay for bacitracin under Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.

Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment

DEFINITION

Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment contains the equivalent of NLT 90.0% and NMT 140.0% of the labeled amounts of neomycin, polymyxin B, and bacitracin.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201BNP): Meets the requirements

ASSAY

• NEOMYCIN

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of Buffer B.3. Combine the aqueous extracts, and dilute with Buffer B.3 to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the Sample solution with Buffer B.3 to obtain a Test Dilution having a neomycin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–140.0%

• POLYMYXIN B

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of Buffer B.6. Combine the aqueous extracts, and dilute with Buffer B.6 to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the Sample solution with Buffer B.6 to obtain a Test Dilution having a concentration that is nominally equivalent to the median level of the standard (10 Polymyxin B Units/mL). Add to each Test Dilution of the standard, a quantity of USP Neomycin Sulfate RS, dissolved in Buffer B.6, to obtain the same concentration of neomycin as in the Test Dilution of the sample.

Acceptance criteria: 90.0%–140.0%

• BACITRACIN

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the Sample solution with Buffer B.1 to obtain a Test Dilution having a concentration that is nominally equivalent to

the median level of the standard (1.0 Bacitracin Unit/mL). If the Sample solution has a concentration of less than 100 Bacitracin Units/mL, add hydrochloric acid to each Test Dilution of the standard to obtain the same concentration of hydrochloric acid as in the Test Dilution of the sample.

Acceptance criteria: 90.0%–140.0%

SPECIFIC TESTS

- **STERILITY TESTS** (71): Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
USP Bacitracin Zinc RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ointment

» Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.

USP Reference standards (11)—

USP Bacitracin Zinc RS
USP Hydrocortisone RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Identification—

A: It meets the requirements under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: The retention time of the major peak for hydrocortisone in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay for hydrocortisone.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for neomycin, Assay for polymyxin B, and Assay for bacitracin—Proceed with Ointment as directed in the Assay for neomycin, in the Assay for polymyxin B, and in the Assay for bacitracin under Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.

Assay for hydrocortisone—Proceed with the Ointment as directed in the Assay for hydrocortisone under Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment.

Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment

DEFINITION

Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment is a sterile ointment containing Neomycin Sulfate, Polymyxin B Sulfate, Bacitracin Zinc, and Hydrocortisone. It contains the equivalent of NLT 90.0% and NMT 140.0% of the labeled amounts of neomycin, polymyxin B, and bacitracin, and NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201BNP):** Meets the requirements
- **B.** The retention time of the hydrocortisone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay for Hydrocortisone.

ASSAY

• NEOMYCIN

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–140.0%

• POLYMYXIN B

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6*. Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 polymyxin B Units/mL). Add to each *Test Dilution* of the standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.

Acceptance criteria: 90.0%–140.0%

• BACITRACIN

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (1.0 bacitracin Unit/mL). If the *Sample solution* has a concentration of less than 100 bacitracin Units/mL, add hydrochloric acid to each *Test Dilution* of the standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of the sample.

Acceptance criteria: 90.0%–140.0%

• HYDROCORTISONE

Mobile phase: Methanol, glacial acetic acid, and water (500:1:500)

Diluent: Methanol and water (1:1)

Standard solution: 0.15 mg/mL of USP Hydrocortisone RS in *Diluent*

Sample solution: Transfer 1.5 g of Ophthalmic Ointment to a separator. Add 3 mL of *n*-hexane, and warm

gently on a steam bath with mild agitation until dissolved. Add 7 mL of *n*-hexane, mix by swirling, and extract with four 15-mL portions of *Diluent*. Collect the extracts in a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Filter the solution, rejecting the first 10 mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-μm packing L1

Flow rate: 2 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **STERILITY TESTS (71):** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS (11)**
 - USP Bacitracin Zinc RS
 - USP Hydrocortisone RS
 - USP Neomycin Sulfate RS
 - USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Acetate Ophthalmic Ointment

DEFINITION

Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Acetate Ophthalmic Ointment is a sterile ointment containing Neomycin Sulfate, Polymyxin B Sulfate, Bacitracin Zinc, and Hydrocortisone Acetate. It contains the equivalent of NLT 90.0% and NMT 140.0% of the labeled amounts of neomycin, polymyxin B, and bacitracin, and NLT 90.0% and NMT 110.0% of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201BNP):** Meets the requirements
- **B.** The retention time of the hydrocortisone acetate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay for Hydrocortisone Acetate.

ASSAY• **NEOMYCIN**(See *Antibiotics—Microbial Assays* (81).)**Sample solution:** Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.**Analysis:** Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.**Acceptance criteria:** 90.0%–140.0%• **POLYMYXIN B**(See *Antibiotics—Microbial Assays* (81).)**Sample solution:** Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6*. Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.**Analysis:** Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 polymyxin B Units/mL). Add to each *Test Dilution* of the standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.**Acceptance criteria:** 90.0%–140.0%• **BACITRACIN**(See *Antibiotics—Microbial Assays* (81).)**Sample solution:** Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.**Analysis:** Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (1.0 bacitracin Unit/mL). If the *Sample solution* has a concentration of less than 100 bacitracin Units/mL, add hydrochloric acid to each *Test Dilution* of the standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of the sample.**Acceptance criteria:** 90.0%–140.0%• **HYDROCORTISONE ACETATE****Mobile phase:** Butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (475:475:70:35:30)**Standard solution:** 0.10 mg/mL of USP Hydrocortisone Acetate RS in water-saturated chloroform**Sample solution:** Nominally 0.10 mg/mL of hydrocortisone acetate from Ophthalmic Ointment prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 2.5 mg of hydrocortisone acetate to a closable container. Add 25.0 mL of water-saturated chloroform and about 10 glass beads. Securely close the container, and shake vigorously for approximately 15 min. Centrifuge, and use the clear, lower chloroform layer.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 3.9-mm × 30-cm; 10-μm packing L3**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of hydrocortisone acetate (C₂₃H₃₂O₆) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Hydrocortisone Acetate RS in the *Standard solution* (mg/mL) C_U = nominal concentration of hydrocortisone acetate in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**SPECIFIC TESTS**• **STERILITY TESTS** (71): Meets the requirements• **OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests* (771).**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.• **USP REFERENCE STANDARDS** (11)

USP Bacitracin Zinc RS

USP Hydrocortisone Acetate RS

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Lidocaine Ointment

» Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Lidocaine Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lidocaine (C₁₄H₂₂N₂O).

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.**USP Reference standards** (11)—

USP Bacitracin Zinc RS

USP Lidocaine RS

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

Identification—**A:** It meets the requirements under *Thin-Layer Chromatographic Identification Test* (201BNP).**B:** The retention time of the major peak for lidocaine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for lidocaine*.**Minimum fill** (755): meets the requirements.**Water Determination, Method I** (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.**Assay for neomycin**—Proceed with Ointment as directed in the *Assay for neomycin* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment*.**Assay for polymyxin B**—Proceed with Ointment as directed in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment*.**Assay for bacitracin**—Proceed with Ointment as directed in the *Assay for bacitracin* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment*.**Assay for lidocaine**—*Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay for lidocaine* under

Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment.

Assay preparation—Using the Ointment, proceed as directed for *Assay preparation* in the *Assay for lidocaine* under *Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment*.

Procedure—Proceed as directed for *Procedure* in the *Assay for lidocaine* under *Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment*. Calculate the quantity, in mg, of lidocaine ($C_{14}H_{22}N_2O$) in the portion of Ointment taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Lidocaine RS in the *Standard preparation*; and r_U and r_S are the lidocaine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Ointment

DEFINITION

Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Ointment contains the equivalent of NLT 90.0% and NMT 130.0% of the labeled amounts of neomycin and polymyxin B, and NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201BNP): Meets the requirements
- **B.** The retention time of the dexamethasone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Dexamethasone*.

ASSAY

• NEOMYCIN

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment in a separator with 50 mL of ether. Extract with four 20-mL portions of *Buffer B.3*. Combine the aqueous extracts, and dilute with *Buffer B.3* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.3* to obtain a *Test Dilution* having a neomycin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–130.0%

• POLYMYXIN B

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Shake a portion of Ophthalmic Ointment with 50 mL of ether in a separator. Extract with four 25-mL portions of *Buffer B.6*. Combine the aqueous extracts, and dilute with *Buffer B.6* to a suitable volume.

Analysis: Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard (10 polymyxin B units/mL). Add to each *Test Dilution* of the standard, a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6*, to obtain the same concentration of neomycin as in the *Test Dilution* of the sample.

Acceptance criteria: 90.0%–130.0%

• DEXAMETHASONE

Mobile phase: Acetonitrile and water (1 in 3)

Diluent: Acetonitrile and methanol (1:1)

Standard solution: 60 µg/mL of USP Dexamethasone RS in *Diluent*

Sample solution: Nominally 60 µg/mL of dexamethasone from Ophthalmic Ointment in *Diluent* prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 3 mg of dexamethasone to a suitable test tube, and add 15 mL of cyclohexane. Heat in a water bath at $75 \pm 5^\circ$ for 10 min. If the Ophthalmic Ointment is not fully dissolved, heat on a steam bath for about 30 s, place a cap on the test tube, and place on a vortex mixer until all solid material is dissolved. Pass with suction through a medium-porosity, sintered-glass filter. Rinse the test tube twice with 10-mL portions of cyclohexane, passing the rinsings through the filter, and discard the filtrate. Wash the filter with about 10 mL of a mixture of *Diluent*, and collect the filtrate in a 50-mL beaker. Wash the test tube and the filter with several 10-mL portions of *Diluent*, and combine the washings in the 50-mL beaker. Transfer the contents of the beaker to a 50-mL volumetric flask with the aid of *Diluent*, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5- to 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dexamethasone ($C_{22}H_{29}FO_5$) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

- r_U = peak response from the *Sample solution*
- r_S = peak response from the *Standard solution*
- C_S = concentration of USP Dexamethasone RS in the *Standard solution* (µg/mL)
- C_U = nominal concentration of dexamethasone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **STERILITY TESTS** (71): Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** (11)
 - USP Dexamethasone RS
 - USP Neomycin Sulfate RS
 - USP Polymyxin B Sulfate RS

Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Suspension

» Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Suspension contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of

the labeled amount of dexamethasone. It may contain one or more suitable buffers, stabilizers, preservatives, and suspending agents.

Packaging and storage—Preserve in tight, light-resistant containers in a cool place or at controlled room temperature. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards (11)—

USP Dexamethasone RS

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

Identification—Transfer a quantity of Ophthalmic Suspension, equivalent to about 2.5 mg of dexamethasone, to a suitable test tube, add 5 mL of chloroform, mix, and centrifuge. Apply 25 μ L of the lower chloroform layer and 25 μ L of a Standard solution of USP Dexamethasone RS in chloroform containing 500 μ g per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and diethylamine (2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the R_f value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH (791): between 3.5 and 6.0.

Change to read:

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.3* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.6* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. Add to each test dilution of the Standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer B.6* (CN 1-May-2017), to obtain the same concentration of neomycin as is present in the *Test Dilution*.

Assay for dexamethasone—

Mobile phase and Chromatographic system—Proceed as directed in the *Assay for dexamethasone* under *Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Ointment*.

Standard preparation—Dissolve an accurately weighed quantity of USP Dexamethasone RS in *Mobile phase* to obtain a solution having a known concentration of about 0.12 mg per mL.

Assay preparation—Dilute an accurately measured volume of freshly mixed Ophthalmic Suspension quantitatively with *Mobile phase* to obtain a solution containing about 0.12 mg of dexamethasone per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay for dexamethasone* under *Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Ointment*. Calculate the quantity, in mg per mL, of $C_{22}H_{29}FO_5$ in the Ophthalmic Suspension taken by the formula:

$$(CL / D)(r_u / r_s)$$

in which L is the labeled quantity, in mg per mL, of dexamethasone in the Ophthalmic Suspension, D is the concentration, in mg per mL, of dexamethasone in the *Assay preparation* based on the labeled quantity in the Ophthalmic Suspension and the extent of dilution, and the other terms are as defined therein.

Neomycin and Polymyxin B Sulfates and Gramicidin Cream

» Neomycin and Polymyxin B Sulfates and Gramicidin Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and gramicidin.

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.

USP Reference standards (11)—

USP Gramicidin RS

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

Minimum fill (755): meets the requirements.

Assay for neomycin and Assay for polymyxin B—Proceed with Cream as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment*.

Assay for gramicidin—Proceed with Cream as directed in the *Assay for gramicidin* under *Neomycin Sulfate and Gramicidin Ointment*.

Neomycin and Polymyxin B Sulfates and Gramicidin Ophthalmic Solution

» Neomycin and Polymyxin B Sulfates and Gramicidin Ophthalmic Solution is a sterile, isotonic aqueous solution of Neomycin Sulfate, Polymyxin B Sulfate, and Gramicidin. It contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and gramicidin.

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards (11)—

USP Gramicidin RS

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

Thin-layer chromatographic identification test

(201BNP): meets the requirements.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH (791): between 4.7 and 6.0.

Change to read:

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with **Buffer B.3** (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with **Buffer B.6** (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. Add to each test dilution of the Standard a quantity of USP Neomycin Sulfate RS, dissolved in **Buffer B.6** (CN 1-May-2017), to obtain the same concentration of neomycin as is present in the *Test Dilution*.

Assay for gramicidin—Proceed as directed for gramicidin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with alcohol to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin and Polymyxin B Sulfates, Gramicidin, and Hydrocortisone Acetate Cream

» Neomycin and Polymyxin B Sulfates, Gramicidin, and Hydrocortisone Acetate Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and gramicidin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Gramicidin RS
USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Minimum fill (755): meets the requirements.

Assay for neomycin and Assay for polymyxin B—Proceed with Cream as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment*.

Assay for gramicidin—Proceed with Cream as directed in the *Assay for gramicidin* under *Neomycin Sulfate and Gramicidin Ointment*.

Assay for hydrocortisone acetate—Proceed with Cream as directed in the *Assay* under *Hydrocortisone Acetate Lotion*.

Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution

» Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution is a sterile solution containing the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone. It may contain one or more suitable buffers, dispersants, and solvents.

Packaging and storage—Preserve in tight, light-resistant containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards (11)—

USP Hydrocortisone RS
USP Neomycin Sulfate RS
USP Polymyxin B Sulfate RS

Sterility Tests (71): meets the requirements.

pH (791): between 2.0 and 4.5.

Change to read:

Assay for neomycin—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Otic Solution diluted quantitatively and stepwise with **Buffer B.3** (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (1.0 µg of neomycin per mL).

Change to read:

Assay for polymyxin B—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Otic Solution diluted quantitatively and stepwise with **Buffer B.6** (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (10 Polymyxin B Units per mL). Add to each test dilution of the Standard a quantity of Neomycin Standard, dissolved in **Buffer B.6** (CN 1-May-2017), to obtain the same concentration of neomycin present in the *Test Dilution*.

Assay for hydrocortisone—

Mobile phase, Standard preparation, and Chromatographic system—Prepare as directed in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment*.

Assay preparation—Transfer 3.0 mL of Otic Solution to a 200-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment*. Calculate the quantity, in mg, of $C_{21}H_{30}O_5$ in each mL of the Otic Solution taken by the formula:

$$(66.67C)(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone RS in the *Standard preparation*, and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin and Polymyxin B Sulfates and Hydrocortisone Ophthalmic Suspension

» Neomycin and Polymyxin B Sulfates and Hydrocortisone Ophthalmic Suspension is a sterile, aqueous suspension containing the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and of polymyxin B. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone.

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards (11)—

USP Hydrocortisone RS

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

Thin-layer chromatographic identification test

(201BNP): meets the requirements.

Sterility Tests (71): meets the requirements.

pH (791): between 4.1 and 7.0.

Change to read:

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.3* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.6* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. Add to each test dilution of the Standard a quantity of Neomycin Sulfate RS, dissolved in *Buffer B.6* (CN 1-May-2017), to yield the same concentration of neomycin as is present in the *Test Dilution*.

Assay for hydrocortisone—

Mobile phase, Standard preparation, and Chromatographic system—Prepare as directed in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment*.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, equivalent to about 30 mg of hydrocortisone, to a 200-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. Filter the solution, rejecting the first 10 mL of the filtrate.

Procedure—Proceed as directed for *Procedure* in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment*. Cal-

culate the quantity, in mg, of $C_{21}H_{30}O_5$ in each mL of the Ophthalmic Suspension taken by the formula:

$$200(C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone RS in the *Standard preparation*; V is the volume, in mL, of Ophthalmic Suspension taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Suspension

» Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Suspension is a sterile suspension containing the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and of polymyxin B. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone. It may contain one or more suitable buffers, dispersants, and preservatives.

Packaging and storage—Preserve in tight, light-resistant containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards (11)—

USP Hydrocortisone RS

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

Thin-layer chromatographic identification test

(201BNP): meets the requirements.

Sterility Tests (71): meets the requirements.

pH (791): between 3.0 and 7.0.

Assay for neomycin and Assay for polymyxin B—Using an accurately measured volume of Otic Suspension, freshly mixed and free from air bubbles, proceed as directed in the *Assay for neomycin* and the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution*.

Assay for hydrocortisone—

Mobile phase, Standard preparation, and Chromatographic system—Prepare as directed in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment*.

Assay preparation—Transfer 3.0 mL of Otic Suspension, freshly mixed and free from air bubbles, to a 200-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. Filter the solution, rejecting the first 10 mL of the filtrate.

Procedure—Proceed as directed for *Procedure* in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment*. Calculate the quantity, in mg, of $C_{21}H_{30}O_5$ in each mL of the Otic Suspension taken by the formula:

$$(66.67C)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin and Polymyxin B Sulfates and Hydrocortisone Acetate Cream

» Neomycin and Polymyxin B Sulfates and Hydrocortisone Acetate Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Hydrocortisone Acetate RS

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

Identification—

A: It meets the requirements under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: The retention time of the major peak for hydrocortisone acetate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for hydrocortisone acetate*.

Minimum fill (755): meets the requirements.

Assay for neomycin and Assay for polymyxin B—Proceed with Cream as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates Cream*.

Assay for hydrocortisone acetate—Proceed with Cream as directed in the *Assay* under *Hydrocortisone Acetate Lotion*.

Neomycin and Polymyxin B Sulfates and Hydrocortisone Acetate Ophthalmic Suspension

» Neomycin and Polymyxin B Sulfates and Hydrocortisone Acetate Ophthalmic Suspension is a sterile suspension of Hydrocortisone Acetate in an aqueous solution of Neomycin Sulfate and Polymyxin B Sulfate. It contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$). It may contain suitable buffers, preservatives, and suspending agents.

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards (11)—

USP Hydrocortisone Acetate RS

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH (791): between 5.0 and 7.0.

Assay for neomycin and Assay for polymyxin B—Proceed with Ophthalmic Suspension as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment*.

Assay for hydrocortisone acetate—Proceed with Ophthalmic Suspension as directed in the *Assay* under *Hydrocortisone Acetate Injectable Suspension*.

Neomycin and Polymyxin B Sulfates and Lidocaine Cream

» Neomycin and Polymyxin B Sulfates and Lidocaine Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lidocaine ($C_{14}H_{22}N_2O$).

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.

USP Reference standards (11)—

USP Lidocaine RS

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

Identification—

A: It meets the requirements under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: The retention time of the major peak for lidocaine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for lidocaine*.

Minimum fill (755): meets the requirements.

Assay for neomycin—Proceed with Cream as directed in the *Assay for neomycin* under *Neomycin and Polymyxin B Sulfates Cream*.

Assay for polymyxin B—Proceed with Cream as directed in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates Cream*.

Assay for lidocaine—

Mobile phase, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay for lidocaine* under *Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment*.

Assay preparation—Using Cream, proceed as directed for the *Assay preparation* in the *Assay for lidocaine* under *Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment*.

Procedure—Proceed as directed for *Procedure* in the *Assay for lidocaine* under *Neomycin and Polymyxin B Sulfates, Bacitracin, and Lidocaine Ointment*. Calculate the quantity, in mg, of lidocaine ($C_{14}H_{22}N_2O$) in the portion of Cream taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Lidocaine RS in the *Standard preparation*; and r_U and r_S are the lidocaine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin and Polymyxin B Sulfates and Pramoxine Hydrochloride Cream

» Neomycin and Polymyxin B Sulfates and Pramoxine Hydrochloride Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of pramoxine hydrochloride ($C_{17}H_{27}NO_3 \cdot HCl$).

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.

USP Reference standards (11)—

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

USP Pramoxine Hydrochloride RS

Identification—

A: Thin-Layer Chromatographic Identification Test (201)—

Test solution—Disperse a quantity of Cream, equivalent to about 25 mg of neomycin, with 20 mL of chloroform in a 60-mL separator. Add 0.2 mL of 2.5 N hydrochloric acid, and shake. Allow the layers to separate for about 30 minutes. Discard the lower chloroform layer, and centrifuge the upper aqueous layer. Use a portion of the centrifuged aqueous layer.

Standard solution—Dissolve suitable quantities of USP Neomycin Sulfate RS and USP Polymyxin B Sulfate RS in 0.1 N hydrochloric acid to obtain a solution containing the equivalent of about 3.5 mg of neomycin and 10,000 USP Polymyxin B Units per mL.

Developing solvent system—Dissolve 0.1 g of benzalkonium chloride in a mixture of isopropyl alcohol, water, and ammonium hydroxide (60:40:10).

Procedure—Proceed as directed in the chapter. Place the plate in a chromatographic chamber saturated with *Developing solvent system*, and develop the chromatogram. Dry the plate at 105° for about 10 minutes, spray with a solution of ninhydrin in butyl alcohol (1 in 200), and heat the plate at 105° for about 15 minutes. The R_f values of the two principal spots in the chromatogram obtained from the *Test solution* correspond to those of the two principal spots in the chromatogram obtained from the *Standard solution*.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for pramoxine hydrochloride*.

pH (791)—Transfer 1 g of Cream to a small beaker, add 10 mL of carbon dioxide-free water, and mix: the pH is between 3.3 and 6.0.

Change to read:

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Cream, equivalent to about 3.5 mg of neomycin, blended for 3 to 5 minutes in a high-speed blender with 249 mL of *Buffer B.3* (CN 1-May-2017) and 1 mL of polysorbate 80. Quantitatively dilute an accurately measured volume of this solution with *Buffer B.3* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of neomycin assumed to be equal to the median level of the Standard (1 µg of neomycin per mL).

Change to read:

Assay for polymyxin—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Cream, equivalent to about 10,000 USP Polymyxin B Units, blended for 3 to 5 minutes in a high-speed blender with 199 mL of *Buffer B.6* (CN 1-May-2017) and 1 mL of polysorbate 80. Quantitatively dilute an accurately measured volume of this solution with *Buffer B.6* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard (10 USP Polymyxin B Units per mL).

Assay for pramoxine hydrochloride—

Mobile phase—Dissolve 3.5 g of dibasic potassium phosphate in 1000 mL of water. Prepare a mixture of this solution, acetonitrile, and triethylamine (700:300:2), and adjust with phosphoric acid to a pH of 4.0 ± 0.1 . Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Prepare a solution of USP Pramoxine Hydrochloride RS in methanol to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Transfer an accurately weighed portion of Cream, equivalent to about 10 mg of pramoxine hydrochloride, to a 50-mL volumetric flask, add about 5 mL of chloroform, and sonicate at about 40° to disperse the Cream. Allow to cool to room temperature, dilute with methanol to volume, and mix. Pass a portion of this solution through a glass fiber filter and a PTFE filter having a 0.45-µm porosity, discarding the first few mL of the filtrate.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector, a guard column that contains packing L7, and a 4.6-mm × 25-cm analytical column that contains packing L7. The column is maintained at a constant temperature of about 40°. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of pramoxine hydrochloride ($C_{17}H_{27}NO_3 \cdot HCl$) in each g of Cream taken by the formula:

$$50(C/W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Pramoxine Hydrochloride RS in the *Standard preparation*; W is the weight, in g, of Cream taken to prepare the *Assay preparation*; and r_U and r_S are the peak areas for pramoxine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin and Polymyxin B Sulfates and Prednisolone Acetate Ophthalmic Suspension

» Neomycin and Polymyxin B Sulfates and Prednisolone Acetate Ophthalmic Suspension is a sterile suspension of Prednisolone Acetate in an aqueous solution of Neomycin Sulfate and Polymyxin B Sulfate. It contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amounts of neomy-

cin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone acetate ($C_{23}H_{30}O_6$). It may contain suitable buffers, preservatives, and suspending agents.

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards (11)—

USP Neomycin Sulfate RS

USP Polymyxin B Sulfate RS

USP Prednisolone Acetate RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay for prednisolone acetate* exhibits a major peak for prednisolone acetate, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay for prednisolone acetate*.

Sterility Tests (71): meets the requirements.

pH (791): between 5.0 and 7.0.

Change to read:

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.3* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer B.6* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. Add to each test dilution of the Standard a quantity of Neomycin Sulfate RS, dissolved in *Buffer B.6* (CN 1-May-2017), to obtain the same concentration of neomycin as is present in the *Test Dilution*.

Assay for prednisolone acetate—

Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system—Prepare as directed in the *Assay for prednisolone acetate* under *Neomycin Sulfate and Prednisolone Acetate Ophthalmic Suspension*.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, equivalent to about 2.5 mg of prednisolone acetate, to a suitable container, add 5.0 mL of *Internal standard solution* and about 100 mL of water-saturated chloroform, and shake by mechanical means for about 15 minutes. Allow to separate for about 15 minutes, and use the clear chloroform layer as the *Assay preparation*.

Procedure—Proceed as directed in the *Assay for prednisolone acetate* under *Neomycin Sulfate and Prednisolone Acetate Ophthalmic Suspension*. Calculate the quantity, in mg, of prednisolone acetate ($C_{23}H_{30}O_6$) in each mL of the Ophthalmic Suspension taken by the formula:

$$0.1(C/V)(R_U/R_S)$$

in which C is the concentration, in μg per mL, of USP Prednisolone Acetate RS in the *Standard preparation*, V is the vol-

ume, in mL, of Ophthalmic Suspension taken, and R_U and R_S are the peak response ratios of prednisolone acetate to betamethasone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate and Prednisolone Acetate Ointment

» Neomycin Sulfate and Prednisolone Acetate Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone acetate ($C_{23}H_{30}O_6$).

Packaging and storage—Preserve in collapsible tubes or in tight containers, protected from light.

USP Reference standards (11)—

USP Neomycin Sulfate RS

USP Prednisolone Acetate RS

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: The retention time of the major peak for prednisolone acetate in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay for prednisolone acetate*.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for neomycin—Proceed with Ointment as directed in the *Assay under Neomycin Sulfate Ointment*.

Assay for prednisolone acetate—

Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system—Prepare as directed in the *Assay for prednisolone acetate* under *Neomycin Sulfate and Prednisolone Acetate Ophthalmic Suspension*.

Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 1 mg of prednisolone acetate, to a suitable container, add 2.0 mL of *Internal standard solution*, dilute with water-saturated chloroform to about 35 mL, and shake to dissolve the ointment. Transfer about 5 mL of this solution to a suitable container, and evaporate to dryness. Add about 5 mL of water-saturated chloroform, and sonicate for 5 minutes. Filter, and use the clear solution as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay for prednisolone acetate* under *Neomycin Sulfate and Prednisolone Acetate Ophthalmic Suspension*. Calculate the quantity, in mg, of prednisolone acetate ($C_{23}H_{30}O_6$) in the portion of Ointment taken by the formula:

$$0.04C(R_U/R_S)$$

in which C is the concentration, in μg per mL, of USP Prednisolone Acetate RS in the *Standard preparation*, and R_U and R_S are the peak response ratios of prednisolone acetate to betamethasone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate and Prednisolone Acetate Ophthalmic Suspension

» Neomycin Sulfate and Prednisolone Acetate Ophthalmic Suspension contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone acetate ($C_{23}H_{30}O_6$).

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards (11)—

USP Neomycin Sulfate RS

USP Prednisolone Acetate RS

Identification—

A: Filter a portion of Ophthalmic Suspension, freshly mixed but free from air bubbles, equivalent to about 60 mg of prednisolone acetate, discarding the filtrate. Wash the filter with about 10 mL of water, and dry at 105° for 3 hours: the IR absorption spectrum of a potassium bromide dispersion of the dried residue on the filter so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Prednisolone Acetate RS.

B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay for prednisolone acetate* exhibits a major peak for prednisolone acetate, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay for prednisolone acetate*.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

pH (791): between 5.5 and 7.5.

Assay for neomycin—Proceed with Ophthalmic Suspension as directed in the *Assay for neomycin under Neomycin and Polymyxin B Sulfates and Prednisolone Acetate Ophthalmic Suspension*.

Assay for prednisolone acetate—

Mobile phase—Prepare a solution containing *n*-butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6).

Internal standard solution—Prepare a solution of betamethasone in tetrahydrofuran containing 10 mg per mL. Dilute this solution with water-saturated chloroform, and mix to obtain a solution having a concentration of about 1 mg per mL.

Standard preparation—Dissolve about 5 mg of USP Prednisolone Acetate RS, accurately weighed, in 10.0 mL of *Internal standard solution*. Use sonication, if necessary, dilute with water-saturated chloroform to 200.0 mL, and mix to obtain a solution having a known concentration of about 25 µg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, equivalent to about 2.5 mg of prednisolone acetate, to a suitable container, add 5.0 mL of *Internal standard solution* and about 100 mL of water-saturated chloroform, and shake by mechanical means for about 15 minutes. Allow to separate for about 15 minutes, and use the clear chloroform layer as the *Assay preparation*.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as di-

rected for *Procedure*: the resolution, *R*, between the analyte and internal standard peaks is not less than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 1.6 for betamethasone and 1.0 for prednisolone acetate. Calculate the quantity, in mg, of prednisolone acetate ($C_{23}H_{30}O_6$) in each mL of the Ophthalmic Suspension taken by the formula:

$$0.1(C/V)(R_U/R_S)$$

in which *C* is the concentration, in µg per mL, of USP Prednisolone Acetate RS in the *Standard preparation*, *V* is the volume, in mL, of Ophthalmic Suspension taken, and *R_U* and *R_S* are the peak response ratios of prednisolone acetate to betamethasone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate and Triamcinolone Acetonide Cream

» Neomycin Sulfate and Triamcinolone Acetonide Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{34}FO_6$).

Packaging and storage—Preserve in collapsible tubes or in tight containers.

USP Reference standards (11)—

USP Neomycin Sulfate RS

USP Triamcinolone Acetonide RS

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test (201BNP)*.

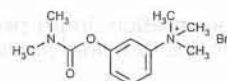
B: Place 2 g of Cream in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification test under Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.

Minimum fill (755): meets the requirements.

Assay for neomycin—Proceed with Cream as directed in the *Assay under Neomycin Sulfate Cream*.

Assay for triamcinolone acetonide—Proceed with Cream as directed in the *Assay under Triamcinolone Acetonide Cream*.

Neostigmine Bromide



$C_{12}H_{19}BrN_2O_2$ 303.20

Benzenaminium, 3-[[[(dimethylamino)carbonyl]oxy]-N,N,N-trimethyl-, bromide.
(*m*-Hydroxyphenyl)trimethylammonium bromide dimethylcarbamate [114-80-7].

» Neostigmine Bromide contains not less than 98.0 percent and not more than 102.0 percent of $C_{12}H_{19}BrN_2O_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Neostigmine Bromide RS

Identification—

A: *Infrared Absorption* (197K).

B: A solution (1 in 50) responds to the tests for Bromide (191).

Melting range (741): between 171° and 176°, with decomposition.

Loss on drying (731)—Dry it at 105° for 3 hours: it loses not more than 2.0% of its weight.

Residue on ignition (281): not more than 0.15%.

Sulfate—Dissolve 250 mg in 10 mL of water, and add 1 mL of 3 N hydrochloric acid and 1 mL of barium chloride TS: no turbidity is produced immediately.

Assay—Dissolve about 750 mg of Neostigmine Bromide, accurately weighed, in a mixture of 70 mL of glacial acetic acid and 20 mL of mercuric acetate TS, add 4 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 30.32 mg of $C_{12}H_{19}BrN_2O_2$.

Neostigmine Bromide Tablets

» Neostigmine Bromide Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{12}H_{19}BrN_2O_2$.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Neostigmine Bromide RS

Identification—Extract a quantity of powdered Tablets, equivalent to about 300 mg of neostigmine bromide, with three 10-mL portions of alcohol, filtering after each extraction. Evaporate the combined filtrates under a stream of nitrogen to dryness. Dissolve the residue in 10 mL of water, transfer to a 125-mL separator with the aid of 5 mL of water, extract with 15 mL of ether, and proceed with the following tests.

A: Evaporate 3 mL of the aqueous layer on a steam bath, under a stream of nitrogen, to dryness. Dissolve the residue, warming if necessary, in 1 mL of alcohol. Add 5 mL of chloroform, filter, evaporate the filtrate under a stream of nitrogen to dryness, and dry the residue at 105° for 30 minutes: the IR absorption spectrum of a potassium bromide dispersion of the residue of neostigmine bromide so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Neostigmine Bromide RS.

B: A portion of the aqueous layer responds to the tests for Bromide (191).

Dissolution, *Procedure for a Pooled Sample* (711)—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Procedure—At the specified time interval, withdraw 30 mL of the solution under test, and filter. Pipet 10 mL each of

the filtered test solution, a Standard solution having a known concentration of USP Neostigmine Bromide RS, and water to provide a blank, into respective 125-mL separators. Proceed as directed for *Procedure* in the Assay, beginning with "Add 15 mL of a solution."

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{12}H_{19}BrN_2O_2$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Standard preparation—Dissolve a suitable quantity of USP Neostigmine Bromide RS, accurately weighed, in water, and dilute quantitatively and stepwise with water to obtain a solution having a concentration of about 40 µg per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of neostigmine bromide, to a 100-mL volumetric flask, add about 50 mL of water, shake by mechanical means for about 30 minutes, add water to volume, mix, and filter. Pipet 4 mL of the clear filtrate into a 50-mL volumetric flask, add water to volume, and mix.

Procedure—Pipet 10 mL each of Assay preparation and Standard preparation into respective 125-mL separators, and treat each solution as follows. Add 15 mL of a solution prepared by dissolving 25 mg of hexanitrodiphenylamine in methylene chloride to make 250 mL, without grinding the solid or heating the solution. Then add 10 mL of 5 N sodium hydroxide, and shake vigorously for 30 seconds. Collect the methylene chloride layer in a 100-mL volumetric flask, and extract the aqueous layer with three 15-mL portions of methylene chloride, collecting the methylene chloride extracts in each respective flask. Add methylene chloride to volume, and mix. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 420 nm, with a suitable spectrophotometer, using methylene chloride as the blank. Calculate the quantity, in mg, of $C_{12}H_{19}BrN_2O_2$ in the portion of Tablets taken by the formula:

$$1.25C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Neostigmine Bromide RS in the Standard preparation, and A_U and A_S are the absorbances of the solutions from the Assay preparation and the Standard preparation, respectively.

Neostigmine Methylsulfate

$C_{13}H_{22}N_2O_6S$ 334.39

Benzenaminium, 3-[[[(dimethylamino)carbonyl]oxy]-N,N,N-trimethyl-, methyl sulfate.

(*m*-Hydroxyphenyl)trimethylammonium methyl sulfate dimethylcarbamate [51-60-5].

» Neostigmine Methylsulfate contains not less than 98.0 percent and not more than 102.0 percent of $C_{13}H_{22}N_2O_6S$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Neostigmine Methylsulfate RS

Identification—

A: *Infrared Absorption* (197K).

B: Place about 1 mg in a small porcelain dish, add 2 mL of water and 0.5 mL of sodium hydroxide solution (2 in 5), and evaporate on a steam bath to dryness. Transfer the residue to a small test tube, and quickly heat in a suitable liq-

uid bath to 250°, continuing at that temperature for about 30 seconds. Cool, dissolve the residue in 0.5 mL of water, cool in ice water, and add 1 mL of diazobenzenesulfonic acid TS: a cherry-red color is produced.

C: Mix about 20 mg with 500 mg of sodium carbonate, and heat the mixture to fusion in a small crucible. Boil the fused mass with 10 mL of water until disintegrated, and filter. Add a few drops of bromine TS to the filtrate, heat to boiling, acidify with hydrochloric acid, and expel the excess bromine by boiling: the resulting solution responds to the tests for *Sulfate* (191).

Melting range (741): between 144° and 149°, determined after drying at 105° for 3 hours.

Loss on drying (731): Dry about 300 mg, accurately weighed, at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.1%.

Chloride—To 10 mL of a solution (1 in 50) add 1 mL of 2 N nitric acid and 1 mL of silver nitrate TS: no opalescence is produced immediately.

Sulfate ion—To 10 mL of a solution (1 in 50) add 1 mL of 3 N hydrochloric acid and 1 mL of barium chloride TS: no turbidity is produced immediately.

Assay—Place about 100 mg of Neostigmine Methylsulfate, accurately weighed, in a 500-mL Kjeldahl flask, dissolve in 150 mL of water, and add 40 mL of 2.5 N sodium hydroxide. Connect the flask by means of a distillation trap to a well-cooled condenser that dips into 25 mL of boric acid solution (1 in 25), distill about 150 mL of the contents of the flask, add methyl purple TS to the solution in the receiver, and titrate with 0.02 N sulfuric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.02 N sulfuric acid is equivalent to 6.688 mg of $C_{13}H_{22}N_2O_6S$.

Neostigmine Methylsulfate Injection

» Neostigmine Methylsulfate Injection is a sterile solution of Neostigmine Methylsulfate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{13}H_{22}N_2O_6S$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, protected from light.

USP Reference standards (11)—
USP Neostigmine Methylsulfate RS

Identification—Transfer a volume of Injection, containing the equivalent of 1 mg of neostigmine methylsulfate, to a small porcelain dish. Evaporate, if necessary, to 2 mL, add 0.5 mL of sodium hydroxide solution (2 in 5), and proceed as directed in *Identification test B* under *Neostigmine Methylsulfate*, beginning with "evaporate on a steam bath."

pH (791): between 5.0 and 6.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Standard preparation—Dissolve a suitable quantity of USP Neostigmine Methylsulfate RS, accurately weighed, in water, and dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 40 µg per mL.

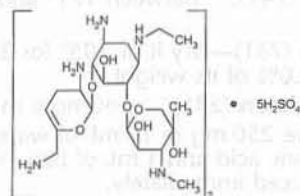
Assay preparation—Pipet an accurately measured volume of Injection, equivalent to about 2 mg of neostigmine methylsulfate, into a 50-mL volumetric flask, add water to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Neostigmine Bromide Tablets*. Calculate the quantity, in mg, of $C_{13}H_{22}N_2O_6S$ in each mL of the Injection taken by the formula:

$$0.05(C/V)(A_U/A_S)$$

in which C is the concentration, in µg per mL, of USP Neostigmine Methylsulfate RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Netilmicin Sulfate



$(C_{21}H_{41}N_5O_7)_2 \cdot 5H_2SO_4$ 1441.55

D-Streptamine, O-3-deoxy-4-C-methyl-3-(methylamino)-β-L-arabinopyranosyl-(1→6)-O-[2,6-diamino-2,3,4,6-tetradeoxy-α-D-glycero-hex-4-enopyranosyl-(1→4)]-2-deoxy-N¹-ethyl-, sulfate (2:5) (salt).

O-3-Deoxy-4-C-methyl-3-(methylamino)-β-L-arabinopyranosyl-(1→4)-O-[2,6-diamino-2,3,4,6-tetradeoxy-α-D-glycero-hex-4-enopyranosyl-(1→6)]-2-deoxy-N¹-ethyl-L-streptamine sulfate (2:5) (salt) [56391-57-2].

» Netilmicin Sulfate has a potency equivalent to not less than 595 µg of netilmicin ($C_{21}H_{41}N_5O_7$) per mg, calculated on the dried basis. [NOTE—Netilmicin Sulfate is extremely hygroscopic. Protect from exposure to moisture.]

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Netilmicin Sulfate RS

USP Sisomicin Sulfate RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: It responds to the tests for *Sulfate* (191).

Specific rotation (781S): between +88° and +96°.

Test solution: 30 mg per mL, in water.

pH (791): between 3.5 and 5.5, in a solution containing 40 mg of netilmicin per mL.

Loss on drying (731)—Dry about 100 mg in vacuum at a pressure not exceeding 5 mm of mercury at 110° for 3 hours: it loses not more than 15.0% of its weight.

Residue on ignition (281): not more than 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

Chromatographic purity—

Dilute phosphoric acid, Mobile phase, Resolution solution, Assay preparation, and Chromatographic system—Proceed as directed in the *Assay*.

Test solution—Use the Assay preparation.

Reference solution—Transfer 1.0 mL of the Test solution to a 100-mL volumetric flask, dilute with Mobile phase to volume, and mix.

Procedure—Separately inject equal volumes (about 20 μ L) of the Test solution and the Reference solution into the chromatograph, and measure the area responses for all the peaks, except those due to the solvent. Calculate the percentage of each impurity in the portion of Netilmicin Sulfate taken by the formula:

$$(r_i / r_s)$$

in which r_i is the peak response of each impurity in the chromatogram obtained from the Test solution, and r_s is the netilmicin peak response in the chromatogram obtained from the Reference solution: not more than 1% of any individual impurity is found, and not more than 5% of total impurities is found.

Assay—

Dilute phosphoric acid—Dilute 5.0 mL of phosphoric acid with water to 1000 mL, and mix.

Mobile phase—Dissolve 20.22 g of sodium 1-heptanesulfonate in Dilute phosphoric acid, dilute with Dilute phosphoric acid to 1000 mL, and mix. To 620 mL of this solution add 380 mL of acetonitrile, mix, and pass through a filter having a 0.45- μ m porosity. Make adjustments if necessary (see System Suitability under Chromatography (621)).

Resolution solution—Prepare a solution in Mobile phase containing about 1 mg of USP Netilmicin Sulfate RS and 1 mg of USP Sisomicin Sulfate RS per mL.

Standard preparation—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of USP Netilmicin Sulfate RS in Mobile phase to obtain a solution having a known concentration of about 1 mg per mL.

Assay preparation—[NOTE—Use low-actinic glassware.] Transfer about 50 mg of Netilmicin Sulfate, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute with Mobile phase, to volume, and mix.

Chromatographic system (see Chromatography (621))—The chromatograph is equipped with a 205-nm detector and a 4.6-mm \times 25-cm column that contains 5- μ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the Resolution solution, and record the peak responses as directed for Procedure: the resolution, R , between sisomicin and netilmicin is not less than 1. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the column efficiency is not less than 3000 theoretical plates; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 1%.

Procedure—Separately inject equal volumes (about 20 μ L) of the Standard preparation and the Assay preparation into the chromatograph, and measure the area responses for the major peaks. Calculate the quantity, in μ g, of netilmicin ($C_{21}H_{41}N_5O_7$) per mg of Netilmicin Sulfate taken by the formula:

$$(W_s P / W_u)(r_u / r_s)$$

in which W_s is the dry weight, in mg, of USP Netilmicin Sulfate RS taken to prepare the Standard preparation; P is the designated potency, in μ g of netilmicin ($C_{21}H_{41}N_5O_7$) per mg, of USP Netilmicin Sulfate RS; W_u is the dry weight, in mg, of Netilmicin Sulfate taken to prepare the Assay preparation; and r_u and r_s are the netilmicin peak responses obtained from the Assay preparation and the Standard preparation, respectively.

Netilmicin Sulfate Injection

» Netilmicin Sulfate Injection is a sterile solution of Netilmicin Sulfate in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of netilmicin ($C_{21}H_{41}N_5O_7$). It may contain one or more suitable buffers, chelating agents, and preservatives.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards (11)—

USP Endotoxin RS

USP Netilmicin Sulfate RS

USP Sisomicin Sulfate RS

Identification—It responds to Identification test A under Netilmicin Sulfate.

Bacterial Endotoxins Test (85)—It contains not more than 1.25 USP Endotoxin Units per mg of netilmicin.

Sterility Tests (71)—It meets the requirements when tested as directed for Membrane Filtration under Test for Sterility of the Product to be Examined.

pH (791): between 3.5 and 6.0.

Particulate Matter in Injections (788): meets the requirements under small-volume injections.

Other requirements—It meets the requirements under Injections and Implanted Drug Products (1).

Assay—

Dilute phosphoric acid, Mobile phase, Resolution solution, Standard preparation, and Chromatographic system—Proceed as directed in the Assay under Netilmicin Sulfate.

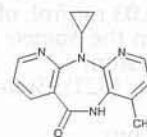
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of netilmicin, to a low-actinic, 100-mL volumetric flask. Dilute with Mobile phase to volume, and mix.

Procedure—Proceed as directed for Procedure in the Assay under Netilmicin Sulfate. Calculate the quantity, in mg, of netilmicin ($C_{21}H_{41}N_5O_7$) in each mL of Injection taken by the formula:

$$0.1(W_s P / 50V)(r_u / r_s)$$

in which V is the volume, in mL, of Injection taken to prepare the Assay preparation, and the other terms are as defined therein.

Nevirapine



$C_{15}H_{14}N_4O$	266.30
6 <i>H</i> -Dipyrido[3,2- <i>b</i> :2',3'- <i>e</i>][1,4]diazepin-6-one, 11-cyclopropyl-5,11-dihydro-4-methyl-; 11-Cyclopropyl-5,11-dihydro-4-methyl-6 <i>H</i> -dipyrido[3,2- <i>b</i> :2', 3'- <i>e</i>][1,4]diazepin-6-one [129618-40-2].	
Hemihydrate	275.31

DEFINITION

Nevirapine is anhydrous or contains one-half molecule of water of hydration. It contains NLT 98.0% and NMT 102.0% of $C_{15}H_{14}N_4O$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K): Do not dry the specimens.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY**• PROCEDURE**

Buffer: 0.025 M of monobasic ammonium phosphate in water prepared as follows. Dissolve 2.9 g of monobasic ammonium phosphate in 800 mL of water. Adjust with 1 N sodium hydroxide to a pH of 5.0, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (1:4)

Standard stock solution A: 0.24 mg/mL of USP Nevirapine Anhydrous RS prepared as follows. Dissolve a quantity of USP Nevirapine Anhydrous RS in Acetonitrile and *Mobile phase* (1:20). Sonicate for at least 15 min, allow to cool to room temperature, and dilute with *Mobile phase* to volume. [NOTE—Do not use after 78 h.]

Standard stock solution B: 0.24 mg/mL of USP Nevirapine Related Compound A RS prepared as follows. Dissolve a quantity of USP Nevirapine Related Compound A RS in a volume of a mixture of acetonitrile and *Mobile phase* (1:3). Sonicate for at least 15 min, allow to cool to room temperature, and dilute with *Mobile phase* to volume.

Standard stock solution C: 0.06 mg/mL of USP Nevirapine Related Compound B RS prepared as follows. Dissolve a quantity of USP Nevirapine Related Compound B RS in a volume of a mixture of acetonitrile and *Mobile phase* (10:22). Sonicate for at least 30 min, allow to cool to room temperature, and dilute with *Mobile phase* to volume.

System suitability solution: 0.03 mg/mL each of USP Nevirapine Anhydrous RS and USP Nevirapine Related Compound A RS and 0.015 mg/mL of USP Nevirapine Related Compound B RS from suitable volumes of *Standard stock solution A*, *Standard stock solution B*, and *Standard stock solution C*, respectively, in *Mobile phase*. [NOTE—Do not use after 78 h.]

Standard solution: 0.03 mg/mL of USP Nevirapine Anhydrous RS in *Mobile phase* from *Standard stock solution A*. [NOTE—Do not use after 78 h.]

Sample stock solution: 0.24 mg/mL of Nevirapine in *Mobile phase* prepared as follows. Transfer the required amount of Nevirapine to a suitable volumetric flask, and add 4% of the final volume with acetonitrile and 80% of the final volume with *Mobile phase*. Sonicate for at least 15 min, allow to cool to room temperature, and dilute with *Mobile phase* to volume.

Sample solution: 0.03 mg/mL of nevirapine anhydrous in *Mobile phase* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-μm packing L60

Column temperature: 35°

Flow rate: 1 mL/min

Injection size: 25 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are shown in Table 1.]

Suitability requirements

Resolution: NLT 5.0 between nevirapine related compound B and nevirapine, and NLT 7.4 between nevirapine and nevirapine related compound A; *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nevirapine ($C_{15}H_{14}N_4O$) in the portion of Nevirapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)

C_U = concentration of Nevirapine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 10 ppm (Official 1-Jan-2018)

• ORGANIC IMPURITIES

Buffer, **Mobile phase**, **System suitability solution**, and **Standard stock solution A:** Proceed as directed in the Assay.

Standard solution: 0.2 μg/mL of USP Nevirapine Anhydrous RS in *Mobile phase* from *Standard stock solution A*

Sample solution: 0.24 mg/mL of Nevirapine in *Mobile phase* prepared as follows. Transfer the required amount of Nevirapine to a suitable volumetric flask, and add 4% of the final volume with acetonitrile and 80% of the final volume with *Mobile phase*. Sonicate for at least 15 min, allow to cool to room temperature, and dilute with *Mobile phase* to volume.

Chromatographic system: Proceed as directed in the Assay, except for *Run time* and *Injection size*.

Run time: At least 80 min

Injection size: 25 μL for *System suitability*; 50 μL for *Analysis*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are shown in Table 1.]

Suitability requirements

Resolution: NLT 5.0 between nevirapine related compound B and nevirapine, and NLT 7.4 between nevirapine and nevirapine related compound A; *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Nevirapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of nevirapine from the *Standard solution*

C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (μg/mL)

C_U = concentration of nevirapine anhydrous in the *Sample solution* (μg/mL)

F = relative response factor for each impurity (see Table 1)

Acceptance criteria See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Nevirapine related compound B	0.7	1.3	0.2
Nevirapine	1.0	1.0	—
Nevirapine related compound A	1.5	1.0	0.2
Nevirapine impurity C	2.8	1.0	0.2
Any other individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.6

SPECIFIC TESTS

• WATER DETERMINATION, Method I (921)

For Nevirapine anhydrous: NMT 0.2%

For Nevirapine hemihydrate: 3.1%–3.9%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **LABELING:** Label to indicate whether it is anhydrous or the hemihydrate.

• USP REFERENCE STANDARDS (11)

USP Nevirapine Anhydrous RS

USP Nevirapine Hemihydrate RS

USP Nevirapine Related Compound A RS

5,11-Dihydro-6H-11-ethyl-4-methyl-dipyrido[3,2-*b*:2',3'-*e*][1,4]diazepin-6-one.

C₁₄H₁₄N₄O 254.29

USP Nevirapine Related Compound B RS

5,11-Dihydro-4-methyl-6H-dipyrido[3,2-*b*:2',3'-*e*][1,4]diazepin-6-one.

C₁₂H₁₀N₄O 226.23

Nevirapine Oral Suspension

DEFINITION

Nevirapine Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of nevirapine (C₁₅H₁₄N₄O).

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHY (201)

Standard solution: 5 mg/mL of USP Nevirapine Anhydrous RS in chloroform

Sample solution: Transfer a volume of Oral Suspension, equivalent to 10 mg of nevirapine, to an 8-mL glass stoppered tube. Pipet 2.0 mL of chloroform into the tube. Shake the solution and allow the two phases to separate; then, using a disposable glass Pasteur pipet, remove some of the organic layer from the bottom, and transfer it to another container.

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel 60 F254

Application volume: 5 µL

Developing solvent system: Ethyl acetate, isopropanol, and concentrated ammonium hydroxide (18:2:0.1)

Spray reagent: 1.35 g of ferric chloride in 25 mL of water and 1.64 g of potassium ferricyanide in 25 mL of water. Mix the two solutions immediately before use.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop in a chamber saturated with a solvent system until the solvent front has moved 6–7 cm from the point of application. Remove the plate from the chamber, mark the solvent front, and dry. Examine under UV light at 254 nm, and outline the spots with a soft pencil. Spray the plate with *Spray reagent*.

Acceptance criteria: The *R_f* value (approximately 0.4–0.5) of the principal blue spot, under UV and after spraying, from the *Sample solution*, corresponds to that from the *Standard solution*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Diluent: Methanol and water (1:4)

Solution A: 13.6 g of monobasic potassium phosphate in 1900 mL of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL.

Solution B: Acetonitrile and *Solution A* (3:97)

Solution C: Acetonitrile and *Solution A* (24:76)

Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
1	100	0
31	0	100
32	100	0
42	100	0

Standard stock solution: Dissolve 50 mg of USP Nevirapine Anhydrous RS in 20 mL of methanol in a 50-mL volumetric flask. Sonicate with intermittent swirling until the sample dissolves. Add water to 1 cm below the meniscus, cool to room temperature, and dilute with water to volume. The concentration is 1 mg/mL of nevirapine.

Standard solution: 0.3 mg/mL of nevirapine from the *Standard stock solution* diluted with *Diluent*

Stock impurity solution: 3 mg of USP Nevirapine Related Compound A RS and 3 mg of USP Nevirapine Related Compound B RS in 20 mL of methanol in a 100-mL volumetric flask. Sonicate to dissolve. Add water to 1 cm below the meniscus, cool to room temperature, and dilute with water to volume.

System suitability solution: Transfer 15.0 mL of *Standard stock solution* and 2.0 mL of *Stock impurity solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Weight determination: Using a 1- to 10-mL suitable pipet and a positive displacement tip, withdraw 5.0 mL of Oral Suspension. The sample should be free of air bubbles. Dispense into a tared vial, and record the weight of the Oral Suspension to ±0.1 mg.

Sample solution: Using a 1- to 10-mL suitable pipet and a positive displacement tip, withdraw Oral Suspension equivalent to 60 mg of nevirapine. The sample should be free of air bubbles. Remove the excess Oral Suspension by wiping the outside of the tip carefully so as not to touch the opening of the tip, and deliver the sample into a 200-mL tared volumetric flask. Record the sample weight to the nearest ±0.1 mg. Add 40 mL of methanol, and sonicate for 5 min with intermittent swirling. Add water to 1 cm below the meniscus. Do not shake the flask. Allow the solution to attain room temperature, and dilute with water to volume. Shake the flask gently, and allow to stand for 5 min.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 15-cm; 3.5-μm packing L10**Guard column:** 4.6-mm × 12.5-mm; 5-μm packing L10**Column temperature:** 35°**Flow rate:** 1.5 mL/min**Injection size:** 20 μL**System suitability****Samples:** *Standard solution* and *System suitability solution***Suitability requirements****Resolution:** NLT 3.0 between nevirapine and nevirapine related compound A; NLT 1.7 between nevirapine and nevirapine related compound B, *System suitability solution***Tailing factor:** NMT 1.5 for the nevirapine peak, *System suitability solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Measure the responses for the nevirapine peak. Calculate the percentage of C₁₅H₁₄N₄O in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL) C_U = nominal concentration of the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**• **DISSOLUTION (711)****Medium:** 0.1 N hydrochloric acid; 900 mL**Apparatus 2:** 25 rpm**Time:** 45 min**Analysis:** Determine the amount of C₁₅H₁₄N₄O dissolved by using the following method.**Diluent:** Dehydrated alcohol and water (1:1)**Mobile phase:** Acetonitrile and water (23:77)**System suitability solution:** Transfer 10 mg of USP Nevirapine Anhydrous RS and 15 mg of methylparaben to a 250-mL volumetric flask, dissolve with 2 mL of *Diluent*, and dilute with *Medium* to volume.**Standard solution:** Transfer 28 mg of USP Nevirapine Anhydrous RS to a 500-mL volumetric flask, add 2 mL of *Diluent*, and sonicate for 1 min. The Standard will not be completely dissolved at this point. Dilute with *Medium* to volume, and visually examine the solution to ensure that the Standard is completely dissolved. The final concentration is 0.056 mg/mL of nevirapine.**Sample solution:** For sample mixing, gently shake the bottle for approximately 10 s by inverting it slowly and rotating it from side to side. The sample should be free of air bubbles. Do not sonicate the sample. Using a 1- to 10-mL suitable positive displacement pipet set at 5 mL, withdraw the equivalent of 50 mg of nevirapine. Remove excess Oral Suspension by wiping the outside of the tip carefully so as not to touch the opening of the tip. Introduce the sample into the dissolution vessel over a period of 1–2 s by immersing the tip of the pipet midway between the paddle and the side of the vessel, approximately 1 cm below the meniscus. Similarly dispense the Oral Suspension into the other vessels. At 45 min, withdraw 5 mL of the solution under test, and pass through a nylon filter of 0.45-μm pore size, discarding the first 2 mL.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 214 nm**Column:** 3.9-mm × 15-cm; 5-μm packing L1**Guard column:** 3.9-mm × 20-mm; packing L1**Flow rate:** 1 mL/min**Injection size:** 10 μL**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 5.0 between nevirapine and methylparaben, *System suitability solution***Tailing factor:** NMT 1.8, *System suitability solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Record the chromatograms for at least 14 min, and measure the responses for the nevirapine peaks.

Calculate the percentage of C₁₅H₁₄N₄O dissolved:

$$\text{Result} = (r_U \times C_S \times V_1) / (r_S \times V_2 \times L) \times 100$$

 r_U = peak response from the *Sample solution* C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL) V_1 = volume of the *Medium*, 900 mL r_S = peak response from the *Standard solution* V_2 = volume of Oral Suspension taken (mL) L = label claim (mg/mL)**Tolerances:** NLT 80% (Q) of the labeled amount of C₁₅H₁₄N₄O is dissolved.**IMPURITIES****Organic Impurities**• **PROCEDURE****Diluent, Solution A, Solution B, Solution C, and Mobile phase:** Prepare as directed in the *Assay*.**Standard stock solution:** Use the *Standard stock solution*, prepared as directed in the *Assay*.**Standard solution:** 0.3 μg/mL of nevirapine from the *Standard stock solution* diluted with *Diluent***System suitability solution:** Prepare as directed in the *Assay*.**Weight determination:** Use the weight obtained as directed for *Weight determination* in the *Assay*.**Sample solution:** Prepare as directed in the *Assay*.**Chromatographic system****Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 15-cm; 3.5-μm packing L10**Column temperature:** 35°**Flow rate:** 1.5 mL/min**Injection size:** 20 μL**System suitability****Samples:** *Standard solution* and *System suitability solution***Suitability requirements****Resolution:** NLT 3.0 between nevirapine and nevirapine related compound A and NLT 1.7 between nevirapine and nevirapine related compound B, *System suitability solution***Tailing factor:** NMT 1.5 for nevirapine, *System suitability solution***Relative standard deviation:** NMT 10.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each unknown impurity in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response for each impurity from the *Sample solution*
 r_S = peak response for nevirapine from the *Standard solution*
 C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of nevirapine in the *Sample solution* (mg/mL)

Acceptance criteria

Individual unknown impurities: NMT 0.1%

Total unknown impurities: NMT 0.2%

[NOTE—The excipients and their degradation products should not be included in the determination of impurities.]

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for absence of *Escherichia coli*. The total aerobic microbial count does not exceed 100 cfu/mL, and the total combined molds and yeasts count does not exceed 50 cfu/mL.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** (11)
 - USP Nevirapine Anhydrous RS
 - USP Nevirapine Related Compound A RS
 5,11-Dihydro-6H-11-ethyl-4-methyl-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one.
 $C_{14}H_{14}N_4O$ 254.29
 - USP Nevirapine Related Compound B RS
 5,11-Dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one.
 $C_{12}H_{10}N_4O$ 226.23

Nevirapine Tablets**DEFINITION**

Nevirapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

Sample: Transfer a portion of powdered Tablets equivalent to 25 mg of nevirapine to a 50-mL volumetric flask. Dissolve in 10 mL of methylene chloride. Swirl the solution for 30–60 s, and pass through a medium sintered-glass, fritted vacuum funnel. Using a glass syringe, pass the filtrate through a Teflon filter of 0.45- μ m pore size. Dry the extract at 105° for a minimum of 1 h.

Acceptance criteria: Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY**PROCEDURE**

Mobile phase: Acetonitrile and water (23:77)
Diluent: Dehydrated alcohol and water (1:1)
System suitability solution: 0.025 mg/mL of USP Nevirapine Anhydrous RS and 0.025 mg/mL of USP Nevirapine Related Compound A RS in *Diluent*
Standard solution: 0.025 mg/mL of USP Nevirapine Anhydrous RS in *Diluent*
Sample stock solution: Nominally 1 mg/mL of nevirapine in *Diluent* prepared as follows. Transfer nevirapine, from finely powdered Tablets (NLT 20), to a suitable size volumetric flask, and add 75% of the final volume with *Diluent*. Sonicate the solution for 20 min,

then shake for 20 min. Cool to room temperature, and dilute with *Diluent* to volume. Centrifuge a portion of the resulting solution at 1500 rpm for 5 min.

Sample solution: Nominally 0.025 mg/mL of nevirapine in *Diluent* from the *Sample stock solution*. Filter a portion of the resulting solution, and discard the first 2 mL of the filtrate.

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 3.9-mm \times 15-cm; packing L1

Flow rate: 1 mL/min

Injection size: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between nevirapine and nevirapine related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nevirapine from the *Sample solution*

r_S = peak response of nevirapine from the *Standard solution*

C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)

C_U = nominal concentration nevirapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)

Medium: 0.1 M phosphate buffer, pH 2.0 (transferring 3.9 mL/L of concentrated phosphoric acid and 5.73 g/L of monobasic sodium phosphate monohydrate in water, adjust with phosphoric acid to a pH of 2.0 ± 0.02); 900 mL

Apparatus 2: 50 rpm. [NOTE—Use stainless steel paddles only. Do not use paddles coated with polytetrafluoroethylene.]

Time: 60 min

Mobile phase, Diluent, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Standard stock solution A: 0.054 mg/mL of USP Nevirapine Anhydrous RS. Add 10% of the final volume with alcohol and 50% of the final volume of *Medium*. Sonicate for 20 min to dissolve, allow to cool to room temperature, and dilute with *Medium* to volume.

Standard stock solution B: 0.028 mg/mL of USP Nevirapine Related Compound A RS. Add 0.8% of the final volume of *Diluent*, sonicate until completely dissolved, and dilute with *Medium* to volume.

Standard solution: 0.014 mg/mL of USP Nevirapine Anhydrous RS from *Standard stock solution A* in *Medium*

System suitability solution: 0.014 mg/mL of USP Nevirapine Anhydrous RS from *Standard stock solution A* and 0.014 mg/mL of USP Nevirapine Related Compound A RS from *Standard stock solution B* in *Medium*

Sample solution: Pass 20 mL of the solution under test through a suitable nylon or glass fiber filter of 0.45- μ m pore size, and dilute with *Medium* to obtain a solution having a final concentration of 0.014 mg/mL of nevirapine.

Analysis

Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/D_U) \times V \times (100/L)$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
 D_U = dilution factor for the *Sample solution*
 V = volume of *Medium*, 900 mL
 L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of nevirapine ($C_{15}H_{14}N_4O$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Sample stock solution, and Sample solution: Proceed as directed in the *Assay*.

Standard solution: 0.125 µg/mL of USP Nevirapine Anhydrous RS from *Standard stock solution A* in *Diluent*

Chromatographic system: Proceed as directed in the *Assay*, except use a run time of at least 13 min.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between nevirapine and nevirapine related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each unknown impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of each unknown impurity from the *Sample solution*
 r_S = peak response of nevirapine from the *Standard solution*
 C_S = concentration of USP Nevirapine Anhydrous RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration nevirapine in the *Sample solution* (mg/mL)

[NOTE—Disregard all peaks due to the solvent or excipients and impurity peaks less than 0.1%.]

Acceptance criteria

Individual unknown impurity: NMT 0.1%

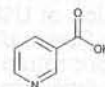
Total unknown impurities: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Nevirapine Anhydrous RS
 USP Nevirapine Related Compound A RS
 5,11-Dihydro-6H-11-ethyl-4-methyl-
 dipyrrodo[3,2-b:2',3'-e][1,4]diazepin-6-one
 $C_{14}H_{14}N_4O$ 254.29

Niacin

$C_6H_5NO_2$ 123.11
 3-Pyridinecarboxylic acid;
 Nicotinic acid [59-67-6].

DEFINITION

Niacin contains NLT 98.0% and NMT 102.0% of niacin ($C_6H_5NO_2$), calculated on the dried basis.

IDENTIFICATION• **A. INFRARED ABSORPTION (197M)**• **B. ULTRAVIOLET ABSORPTION (197U)**

Wavelength range: 200–300 nm

Buffer solution: Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water. Adjust with 50% sodium hydroxide solution to a pH of 7.0.

Sample solution: 20 µg/mL in *Buffer solution*

Acceptance criteria: Meets the requirements. The A_{239}/A_{263} ratio is 0.46–0.52.

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Diluent: Methanol and water (82:18)

Mobile phase: Methanol and water (82:18), adjusted with glacial acetic acid to a pH of 3.15 ± 0.05

System suitability solution: 0.25 mg/mL of USP Niacin RS, 0.050 mg/mL of USP 6-Hydroxynicotinic Acid RS, and 0.10 mg/mL of pyridine in *Diluent*

Standard solution: 0.25 mg/mL of USP Niacin RS in *Diluent*

Sample solution: 0.25 mg/mL of Niacin in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 15-cm; 5-µm packing L8

Flow rate: 1.0 mL/min

Injection volume: 25 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for pyridine, 6-hydroxynicotinic acid, and niacin are about 0.14, 0.64, and 1.0, respectively, *System suitability solution*.]

Suitability requirements

Resolution: NLT 1.5 between pyridine and 6-hydroxynicotinic acid and NLT 1.5 between 6-hydroxynicotinic acid and niacin, *System suitability solution*

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of niacin ($C_6H_5NO_2$) in the portion of Niacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of niacin from the *Sample solution*
 r_S = peak response of niacin from the *Standard solution*
 C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)
 C_U = concentration of Niacin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **CHLORIDE AND SULFATE**, Chloride (221)
Standard: 0.15 mL of 0.020 N hydrochloric acid
Sample: 0.50 g of Niacin
Acceptance criteria: NMT 0.02%
- **CHLORIDE AND SULFATE**, Sulfate (221)
Standard: 0.10 mL of 0.020 N sulfuric acid
Sample: 0.50 g of Niacin
Acceptance criteria: NMT 0.02%

Delete the following:

- **HEAVY METALS**, Method I (231)
Test preparation: Mix 1 g with 4 mL of 1 N acetic acid, and dilute with water to 25 mL. Heat gently until solution is complete, and cool.
Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)
- **RELATED COMPOUNDS**
Solution A: Dissolve 0.6 g of glacial acetic acid in 1 L of water, and adjust with 10% ammonium hydroxide solution to a pH of 5.6.
Solution B: Acetonitrile and methanol (1:1)
Mobile phase: Gradient elution. See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10 ^a	100	0
30	20	80
35	20	80
36	100	0
48	100	0

^a The gradient start time may be adjusted to achieve the required resolution between the 6-methylnicotinic acid and 6,6'-dinicotinic acid peaks of the System suitability solution.

System suitability solution: Transfer 3 mg each of USP 6-Methylnicotinic Acid RS, USP 6,6'-Dinicotinic Acid RS, and pyridine to a 100-mL volumetric flask, dissolve, and dilute with Solution A to volume. Transfer 2.0 mL of the resultant solution to a 5-mL volumetric flask, and dilute with Solution A to volume.

Standard solution: 0.012 mg/mL of USP Niacin RS in Solution A

Sample solution: Transfer 120 mg of Niacin to a 10-mL volumetric flask, add 200 µL of 10% ammonium hydroxide solution, and dilute with Solution A to volume. Shake the flask until the Niacin is completely dissolved.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 25-cm; 4-µm packing L1

Column temperature: 15°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Samples: System suitability solution and Standard solution

[NOTE—The relative retention times for 6-methylnicotinic acid, 6,6'-dinicotinic acid, and pyridine are about 1.0, 1.03, and 1.4, respectively, System suitability solution.]

Suitability requirements

Resolution: NLT 1.5 between 6-methylnicotinic acid and 6,6'-dinicotinic acid peaks, System suitability solution

Relative standard deviation: NMT 10.0% for replicate injections, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Niacin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of each impurity from the Sample solution

r_s = peak response of niacin from the Standard solution

C_s = concentration of USP Niacin RS in the Standard solution (mg/mL)

C_u = concentration of Niacin in the Sample solution (mg/mL)

Acceptance criteria: See Table 2. Disregard any impurity peak less than 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Isocinchomeronic acid	0.38	0.05
6-Hydroxynicotinic acid	0.63	0.05
Isonicotinic acid	0.92	0.05
Niacin	1.00	—
6-Methylnicotinic acid	2.61	0.05
6,6'-Dinicotinic acid	2.68	0.05
5-Nitronicotinic acid	2.76	0.05
Pyridine	3.76	0.05
3-Nitropyridine	3.83	0.05
3,5-Dinitropyridine	4.03	0.05
3-Ethylpyridine	4.72	0.05
5-Ethyl-2-methylpyridine	5.00	0.05
Any individual unspecified impurity	—	0.05
Total impurities	—	0.20

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry at 105° for 1 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• USP REFERENCE STANDARDS (11)

USP 6,6'-Dinicotinic Acid RS
USP 6-Hydroxynicotinic Acid RS
USP 6-Methylnicotinic Acid RS
USP Niacin RS

Niacin Injection

» Niacin Injection is a sterile solution of Niacin and niacin sodium in Water for Injection, made with the aid of Sodium Carbonate or Sodium Hydroxide. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of C₆H₅NO₂.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards (11)—

USP Endotoxin RS

USP Niacin RS

Identification—To a volume of Injection, equivalent to about 100 mg of niacin, add 0.3 mL of 3 N hydrochloric acid, evaporate, if necessary, on a steam bath to about 2 mL, and allow to stand for 1 hour in a cool place. Filter by suction, wash with small volumes of ice-cold water until the last washing does not give a reaction for chloride, and dry at 105° for 1 hour: the niacin so obtained responds to *Identification* tests A and B under *Niacin*.

Bacterial Endotoxins Test (85)—It contains not more than 3.5 USP Endotoxin Units per mg of niacin.

pH (791): between 4.0 and 6.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—Proceed with Injection as directed for *Niacin* or *Niacinamide Assay* (441), *Chemical Method*, using *Standard Niacin Preparation* as the *Standard Preparation* in the *Assay Procedure*, and the following as the *Assay Preparation*. Transfer an accurately measured volume of Injection, equivalent to about 50 mg of niacin, to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, dilute with water to volume, and mix. Calculate the quantity, in mg, of $C_6H_5NO_2$ in each mL of the Injection taken by the formula:

$$(50 / V)(A_U / A_S)$$

in which *V* is the volume, in mL, of Injection taken.

Niacin Tablets

DEFINITION

Niacin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of niacin ($C_6H_5NO_2$).

IDENTIFICATION• **A. INFRARED ABSORPTION** (197M)

Sample: Heat a portion of finely powdered Tablets, equivalent to 500 mg of niacin, with 25 mL of alcohol on a steam bath for a few min. Filter, and wash the residue with a few mL of hot alcohol. To the filtrate add 30 mL of water, and evaporate to 25 mL on the steam bath. Cool, filter if insoluble matter separates, and evaporate the filtrate to 10 mL. Cool, and place in a refrigerator for 1 h. Filter the separated niacin with suction, wash it with a few mL of cold alcohol, and dry at 105° for 1 h.

Acceptance criteria: Meet the requirements

• **B. ULTRAVIOLET ABSORPTION** (197U)

Medium: 6.8 mg/mL of monobasic potassium phosphate in water, adjusted to a pH of 7.0 with 50% sodium hydroxide solution

Sample solution: 20 µg/mL in *Medium* from the *Sample* obtained in the *Identification* test A

Acceptance criteria: Meets the requirements in the chapter. The A_{237}/A_{262} ratio is 0.46–0.50.

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**ASSAY**• **PROCEDURE**

Solution A: 5-mM solution of sodium 1-hexanesulfonate in water

Mobile phase: Methanol, acetonitrile, glacial acetic acid, and *Solution A* (14:7:1:78)

Standard solution: 0.050 mg/mL of USP Niacin RS in water. Dissolve with the aid of heat in a steam bath.

Sample solution: Transfer an equivalent to 500 mg of Niacin from NLT 20 finely powdered Tablets to a suitable flask. Add 50 mL of water, and heat on a steam bath for 30 min. Sonicate for 2 min, shake by mechanical means for 15 min, and cool to room temperature. Dilute with water to 0.050 mg/mL, and filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 262 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 1.3 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates for the analyte peak

Tailing factor: NMT 2.0 for the analyte peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of niacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 60 min

Standard solution: 0.02 mg/mL of USP Niacin RS in the *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: Maximum at about 260 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the concentration of niacin in the *Sample solution* in comparison with the *Standard solution*.

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved:

$$\text{Result} = (C \times D \times V/L) \times 100$$

C = determined concentration of niacin in the *Sample solution* (mg/mL)

D = dilution factor for the *Sample solution*

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 65% (Q) of the labeled amount of niacin ($C_6H_5NO_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**
USP Niacin RS

Niacin Extended-Release Tablets

DEFINITION

Niacin Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of niacin ($C_6H_5NO_2$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Diluent: Methanol and water (82:18)

Mobile phase: Methanol and water (82:18), adjusted with glacial acetic acid to a pH of 3.15 ± 0.05

Standard solution: 250 μ g/mL of USP Niacin RS, 50 μ g/mL of USP 6-Hydroxynicotinic Acid RS, and 97.8 μ g/mL of pyridine in *Diluent*

Sample solution: Transfer a quantity of powder, equivalent to 50 mg of niacin from NLT 20 finely powdered Tablets, to a suitable flask, add *Diluent*, and stir for 2 h. Dilute with *Diluent* to a final concentration of 250 μ g/mL of niacin.

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L8

Flow rate: 1.0 mL/min

Injection volume: 25 μ L

System suitability

Sample: *Standard solution*

[NOTE—See Table 4 for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between pyridine and 6-hydroxynicotinic acid, and NLT 1.5 between 6-hydroxynicotinic acid and niacin

Relative standard deviation: NMT 3.0% for each of the peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of niacin from the *Sample solution*

r_S = peak area of niacin from the *Standard solution*

C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of niacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Test 1

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Times: 1, 3, 6, 9, 12, and 20 h; without *Medium* replacement. [NOTE—Withdraw the same volume at each time point.]

Solution A: Solution of sodium heptanesulfonate in acetic acid, methanol, and water (4:44:33:19), w/w¹

Mobile phase: Mixture of methanol, water, and *Solution A* (560:440:25)

Standard solution: USP Niacin RS at a known concentration in water in the range of 75–750 μ g/mL

Sample solution: Filtered portion of the solution under test suitably diluted with *Medium* if necessary

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 15-cm; 10- μ m packing L1

Flow rate: 1.0 mL/min

Injection volume: 15 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Determine, in mg/mL, the content of niacin ($C_6H_5NO_2$) in the *Medium* at each time point:

$$\text{Result} = (r_U/r_S) \times C_S \times D$$

r_U = peak area of niacin from the *Sample solution*

r_S = peak area of niacin from the *Standard solution*

C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at each time point:

At 1 h:

$$\text{Result}_1 = (C_1 \times V/L) \times 100$$

At 3 h:

$$\text{Result}_2 = [C_2 \times (V - V_3) + C_1 \times V_3] \times 100/L$$

At 6 h:

$$\text{Result}_3 = \{C_3 \times [V - 2 \times V_3] + (C_1 + C_2) \times V_3\} \times 100/L$$

At 9 h:

$$\text{Result}_4 = \{C_4 \times [V - 3 \times V_3] + (C_1 + C_2 + C_3) \times V_3\} \times 100/L$$

At 12 h:

$$\text{Result}_5 = \{C_5 \times [V - 4 \times V_3] + (C_1 + C_2 + C_3 + C_4) \times V_3\} \times 100/L$$

At 20 h:

$$\text{Result}_6 = \{C_6 \times [V - 5 \times V_3] + (C_1 + C_2 + C_3 + C_4 + C_5) \times V_3\} \times 100/L$$

C = as C_1, C_2, \dots, C_6 , the content of niacin in the *Medium* at each time point (mg/mL)

V = volume of *Medium*, 900 mL

V_3 = volume of sample withdrawn at each time point (mL)

L = label claim (mg/Tablet)

Tolerances: The percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at the times specified in Tables 1, 2, and 3 conforms to Acceptance Table 2 in Dissolution (711).

Table 1. For Tablets labeled to contain 500 mg or less/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 15
3	17–32
6	33–48

¹ Commercially available from Waters Corporation as PIC B7 Reagent (Part #85103).

Table 1. For Tablets labeled to contain 500 mg or less/Tablet (Continued)

Time (h)	Amount Dissolved (%)
9	43–63
12	52–77
20	NLT 75

Table 2. For Tablets labeled to contain 750 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 15
3	16–31
6	31–46
9	42–62
12	51–76
20	NLT 75

Table 3. For Tablets labeled to contain 1000 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 15
3	15–30
6	30–45
9	40–60
12	50–75
20	NLT 75

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Acid stage medium: 0.1 N hydrochloric acid; 900 mL
Buffer stage medium: 6.8 g of monobasic potassium phosphate and 0.89 g of sodium hydroxide pellets in 1000 mL of water. Adjust with diluted sodium hydroxide or phosphoric acid to a pH of 6.8; 900 mL.

Apparatus 1: 100 rpm

Times: 1, 4, 12, and 24 h: 1 and 4 h in the *Acid stage medium*; 12 and 24 h in the *Buffer stage medium*. Replace the volume withdrawn with the equal volume of medium preheated to $37 \pm 0.5^\circ$.

Procedure: After 4 h replace the *Acid stage medium* with the *Buffer stage medium*, and run the test for the times specified (additional 20 h for a total of 24 h).

[NOTE—Withdraw the same volume at each time point.

Pass a portion of the solution through a suitable filter.]
Standard stock solution: 0.2 mg/mL of USP Niacin RS in water

Standard solution 1: Dilute *Standard stock solution* with *Acid stage medium* to a final concentration of 0.01 mg/mL of USP Niacin RS.

Standard solution 2: Dilute *Standard stock solution* with *Buffer stage medium* to a final concentration of 0.01 mg/mL of USP Niacin RS.

Sample solution

For Tablets labeled to contain 500 mg: Dilute a filtered portion of the solution under test with appropriate dissolution medium 25-fold.

For Tablets labeled to contain 750 mg: Dilute a filtered portion of the solution under test with appropriate dissolution medium 33-fold.

For Tablets labeled to contain 1000 mg: Dilute a filtered portion of the solution under test with appropriate dissolution medium 50-fold.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 262 nm

Path length: 1 cm

Blank: *Acid stage medium* or *Buffer stage medium*

Analysis

Samples: *Standard solution 1* or *Standard solution 2* and *Sample solution*

Determine the concentration, in mg/mL, of niacin ($C_6H_5NO_2$) in the sample withdrawn from the vessel at each time point:

$$\text{Result} = [(A_U - A_B)/A_S] \times C_S \times D$$

A_U = absorbance of the *Sample solution*

A_B = absorbance of the *Blank*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Niacin RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at each time point:

At 1 h:

$$\text{Result}_1 = (C_1 \times V) \times 100/L$$

At 4 h:

$$\text{Result}_2 = (C_2 \times V + C_1 \times V_3) \times 100/L$$

At 12 h:

$$\text{Result}_3 = [(C_3 + C_2) \times V + C_1 \times V_3] \times 100/L$$

At 24 h:

$$\text{Result}_4 = [(C_4 + C_2) \times V + (C_1 + C_3) \times V_3] \times 100/L$$

C = as C_1, \dots, C_4 , the content of niacin in the related dissolution medium at each time point (mg/mL)

V = volume of *Medium*, 900 mL

V_3 = volume of sample withdrawn at each time point (mL)

L = label claim (mg/Tablet)

Tolerances: The percentage of the labeled amount of niacin ($C_6H_5NO_2$) dissolved at the times specified in *Tables 4* and *5* conforms to *Acceptance Table 2* in *Dissolution* (711).

Table 4. For Tablets labeled to contain 500 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 25
4	30–50
12	65–85
24	NLT 80

Table 5. For Tablets labeled to contain 750 and 1000 mg/Tablet

Time (h)	Amount Dissolved (%)
1	NMT 25
4	30–50
12	55–75
24	NLT 80

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES• **ORGANIC IMPURITIES**

Diluent, Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of 6-hydroxynicotinic acid or pyridine in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of 6-hydroxynicotinic acid or pyridine from the Sample solution

r_S = peak area of 6-hydroxynicotinic acid or pyridine from the Standard solution

C_S = concentration of USP 6-Hydroxynicotinic Acid RS or pyridine in the Standard solution ($\mu\text{g/mL}$)

C_U = nominal concentration of niacin in the Sample solution ($\mu\text{g/mL}$)

Calculate the percentage of any unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each impurity from the Sample solution

r_S = peak area of niacin from the Standard solution

C_S = concentration of USP Niacin RS in the Standard solution ($\mu\text{g/mL}$)

C_U = nominal concentration of niacin in the Sample solution ($\mu\text{g/mL}$)

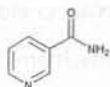
Acceptance criteria: See Table 6.

Table 6

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pyridine	0.14	0.2
6-Hydroxynicotinic acid	0.64	0.2
Niacin	1.0	—
Any unspecified impurity	—	0.1
Total impurities	—	1.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** When more than one Dissolution test is given, the labeling states the Dissolution test used only if Test 1 is not used.
- **USP REFERENCE STANDARDS (11)**
USP 6-Hydroxynicotinic Acid RS
USP Niacin RS

Niacinamide

$\text{C}_6\text{H}_6\text{N}_2\text{O}$
3-Pyridinecarboxamide;
Nicotinamide [98-92-0].

122.12

DEFINITION

Niacinamide contains NLT 98.5% and NMT 101.5% of niacinamide ($\text{C}_6\text{H}_6\text{N}_2\text{O}$), calculated on the dried basis.

IDENTIFICATION• **A. INFRARED ABSORPTION (197K)**• **B. ULTRAVIOLET ABSORPTION (197U)**

Sample solution: 20 $\mu\text{g/mL}$ in water

Acceptance criteria: It meets the requirements in the chapter, and the A_{245}/A_{262} ratio is 0.63–0.67.

ASSAY• **PROCEDURE**

Mobile phase: Methanol and 0.005 M sodium 1-heptanesulfonate (30:70)

Standard solution: Transfer 50 mg of USP Niacinamide RS to a 100-mL volumetric flask. Add 3 mL of water to dissolve, and dilute with Mobile phase to volume. Dilute with Mobile phase to 0.04 mg/mL.

Niacin standard solution: Prepare as directed in the Standard solution, using USP Niacin RS instead of USP Niacinamide RS.

Sample solution: Prepare as directed in the Standard solution, using Niacinamide instead of USP Niacinamide RS.

System suitability solution: Mix equal volumes of the Standard solution and Niacin standard solution.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 2 mL/min

Injection size: 20 μL

System suitability

Samples: Standard solution and System suitability solution

Suitability requirements

Resolution: NLT 3.0 between niacin and niacinamide, System suitability solution

Relative standard deviation: NMT 2.0%, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of niacinamide ($\text{C}_6\text{H}_6\text{N}_2\text{O}$) in the portion of Niacinamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the Sample solution

r_S = peak area from the Standard solution

C_S = concentration of USP Niacinamide RS in the Standard solution (mg/mL)

C_U = concentration of Niacinamide in the Sample solution (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES• **RESIDUE ON IGNITION (281):** NMT 0.1%**Delete the following:**

- **HEAVY METALS, Method II (231):** NMT 30 ppm • (Official 1-

Jan-2018)

• **READILY CARBONIZABLE SUBSTANCES (271)**

Sample solution: Dissolve 200 mg of Niacinamide in 5 mL of sulfuric acid.

Acceptance criteria: The Sample solution has no more color than Matching Fluid A.

SPECIFIC TESTS• **MELTING RANGE OR TEMPERATURE (741):** 128°–131°• **LOSS ON DRYING (731):** Dry a sample over silica gel for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
 - USP Niacin RS
 - USP Niacinamide RS

Niacinamide Injection

» Niacinamide Injection is a sterile solution of Niacinamide in Water for Injection. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of $C_6H_6N_2O$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards (11)—

- USP Niacinamide RS
- USP Endotoxin RS

Identification—Dilute a quantity of the Injection, equivalent to about 200 mg of niacinamide, with water to about 10 mL. Add 1 mL of 2.5 N sodium hydroxide, evaporate on a steam bath to dryness, add 5 mL of water, and similarly evaporate to about 1 mL: during the initial evaporation, the odor of ammonia is perceptible. Neutralize to litmus paper with 3 N hydrochloric acid, add 1 mL of the acid in excess, and place the solution in a refrigerator for 2 hours. Then filter, wash the precipitated niacin with small portions of ice-cold water until free from chloride, and dry at 105° for 1 hour: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Niacinamide RS.

Bacterial Endotoxins Test (85)—It contains not more than 3.5 USP Endotoxin Units per mg of niacinamide.

pH (791): between 5.0 and 7.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products (1)*.

Assay—Proceed with Injection as directed for under *Niacin or Niacinamide Assay (441)*, *Chemical Method*, using *Standard Niacinamide Preparation* as the *Standard Preparation* in the *Assay Procedure*, and the following as the *Assay Preparation*. Dilute an accurately measured volume of Injection, equivalent to about 50 mg of niacinamide, with water to 500 mL in a volumetric flask, and mix. Pipet 10 mL of the solution into a 100-mL volumetric flask, dilute with water to volume, and mix. Calculate the quantity, in mg, of $C_6H_6N_2O$ in each mL of the Injection taken by the formula:

$$(50 / V)(A_U / A_S)$$

in which *V* is the volume, in mL, of Injection taken.

Niacinamide Tablets**DEFINITION**

Niacinamide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of niacinamide ($C_6H_6N_2O$).

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

Sample: Extract a quantity of powdered Tablets, equivalent to 500 mg of niacinamide, with two 10-mL portions of alcohol, evaporate the filtered alcohol extracts on a steam bath, and dry at 80° for 2 h.

Acceptance criteria: Meet the requirements

- **B. ULTRAVIOLET ABSORPTION (197U)**

Sample solution: 20 µg/mL of niacinamide in water from the *Sample* obtained in *Identification test A*

Acceptance criteria: Meets the requirements in the chapter. The ratio A_{245}/A_{262} is 0.63–0.67.

ASSAY

- **NIACIN OR NIACINAMIDE ASSAY, Chemical Method (441)**

Standard niacinamide preparation: Prepare as directed in the chapter.

Assay preparation: Transfer an equivalent of 25 mg of niacinamide, from NLT 10 finely powdered Tablets, to a suitable flask. Add 50 mL of water and heat, if necessary, until no more dissolves. Cool, dilute with water to 10 µg/mL, mix, and filter.

Analysis: Proceed as directed in the chapter for *Procedure*.

Calculate the percentage of the labeled amount of niacinamide ($C_6H_6N_2O$) in the portion of Tablets taken:

$$\text{Result} = (A_U / A_S) \times (C_S / C_U) \times 100$$

A_U = absorbance of the *Assay preparation*

A_S = absorbance of the *Standard niacinamide preparation*

C_S = concentration of the *Standard niacinamide preparation* (µg/mL)

C_U = nominal concentration of niacinamide in the *Assay preparation* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION, Procedure for a Pooled Sample (711)**

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: Known concentration of USP Niacinamide RS in the *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with the *Medium* if necessary

Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 140 mg of sodium 1-hexanesulfonate per 100 mL

Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of niacinamide ($C_6H_6N_2O$) dissolved:

$$\text{Result} = (r_U / r_S) \times (C_S \times D \times V / L) \times 100$$

r_U = peak area of niacinamide from the *Sample solution*

r_S = peak area of niacinamide from the *Standard solution*

C_S = concentration of USP Niacinamide RS in the *Standard solution* (mg/mL)

D = dilution factor for the *Sample solution*

V = volume of *Medium*, 900 mL

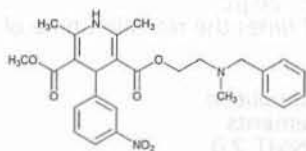
L = label claim (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of niacinamide ($C_6H_6N_2O$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Nicardipine RS

Nicardipine Hydrochloride

$C_{26}H_{29}N_3O_6 \cdot HCl$ 515.99

3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-, methyl 2-[methyl(phenylmethyl)amino]ethyl ester, monohydrochloride; 2-(Benzylmethylamino)ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate monohydrochloride [54527-84-3].

Nicardipine free base

$C_{26}H_{29}N_3O_6$ 479.52
[55985-32-5].

DEFINITION

Nicardipine Hydrochloride contains NLT 98.0% and NMT 102.0% of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (17K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride (191)**
Sample solution: 2.5 mg/mL in methanol
Acceptance criteria: Meets the requirements

ASSAY• **PROCEDURE**

Protect all solutions from light.

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust the solution with potassium hydroxide to a pH of 4.8.

Solution A: Acetonitrile and methanol (82:18)

Mobile phase: *Solution A* and *Buffer* (40:60)

System suitability solution: 0.6 mg/mL of USP Nicardipine Hydrochloride RS in *Mobile phase* prepared as follows. To a suitable amount of USP Nicardipine Hydrochloride RS in a suitable volumetric flask add *Mobile phase* to fill about 10% of the volume of the flask, and sonicate for 2 min. Then add 10% hydrogen peroxide solution to fill an additional 10% of the volume of the flask. Allow it to stand for 30 min, then dilute with *Mobile phase* to volume.

Nicardipine Hydrochloride degrades to produce nicardipine pyridine analog, nicardipine dimethyl ester analog, and nicardipine bis analog. Use a freshly prepared sample to avoid further degradation for analysis.

Standard solution: 0.1 mg/mL of USP Nicardipine Hydrochloride RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Nicardipine Hydrochloride in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 237 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Column temperature: 35°–40°. [NOTE—To attain resolution, the *Column temperature* may be adjusted instead of the *Mobile phase* composition.]

Flow rate: 1.5 mL/min

Injection volume: 50 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$) in the portion of Nicardipine Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of nicardipine from the *Sample solution*

r_s = peak response of nicardipine from the *Standard solution*

C_s = concentration of USP Nicardipine Hydrochloride RS in the *Standard solution* (mg/mL)

C_u = concentration of Nicardipine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm • (Official 1-Jan-2018)

• **ORGANIC IMPURITIES**

Protect all solutions from light.

Buffer, Solution A, Mobile phase, and System suitability solution: Proceed as directed in the *Assay*.

Standard solution: 3 μg/mL of USP Nicardipine Hydrochloride RS in *Mobile phase*

Sample solution: 0.6 mg/mL of Nicardipine Hydrochloride in *Mobile phase*. [NOTE—Sonication may be necessary for complete dissolution.]

Chromatographic system: Proceed as directed in the *Assay*, except for the *Run time*.

Run time: NLT 4 times the retention time of nicardipine

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between nicardipine and nicardipine dimethyl ester analog and NLT 1.5 between nicardipine bis analog and nicardipine dimethyl ester analog, *System suitability solution*

Relative standard deviation: NMT 2%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of the sample taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of each impurity from the *Sample solution*

- r_s = peak response of nicardipine from the *Standard solution*
 C_s = concentration of USP Nicardipine Hydrochloride RS in the *Standard solution* (mg/mL)
 C_u = concentration of Nicardipine Hydrochloride in the *Sample solution* (mg/mL)
Acceptance criteria: See Table 1. Disregard peaks less than 0.01%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nicardipine pyridine analog ^a	0.67	0.1
Nicardipine	1.00	—
Nicardipine dimethyl ester analog ^b	1.20	0.5
Nicardipine bis analog ^c	1.33	0.5
Any other individual unidentified impurity	—	0.1
Total impurities	—	1.0

^a 3-[2-(Benzyl(methyl)amino)ethyl] 5-methyl 2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate dihydrochloride.

^b Dimethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

^c Bis[2-(benzyl(methyl)amino)ethyl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate dihydrochloride.

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry at 105°, protected from light, to constant weight.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tightly closed containers, protected from light.

• USP REFERENCE STANDARDS (11)

USP Nicardipine Hydrochloride RS

Nicardipine Hydrochloride Injection

DEFINITION

Nicardipine Hydrochloride Injection is a sterile solution of Nicardipine Hydrochloride. It contains NLT 90.0% and NMT 110.0% each of the labeled amount of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$) and sorbitol.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Buffer: 1.36 g/L of potassium dihydrogen phosphate in water

Mobile phase: Methanol and Buffer (800:200)

Diluent: Acetonitrile and Buffer (50:50)

Standard solution: 0.1 mg/mL of USP Nicardipine Hydrochloride RS in *Diluent*. Sonication may be used to aid in dissolution. Pass through a suitable filter of 0.45- μ m pore size. Discard the first 2–3 mL of filtrate.

Sample solution: Nominally equivalent to 0.1 mg/mL of nicardipine hydrochloride in *Diluent* from a suitable

volume of Injection. Pass through a suitable filter of 0.45- μ m pore size. Discard the first 2–3 mL of filtrate. [NOTE—*Sample solution* is stable for about 26 h.]

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: NLT 2 times the retention time of nicardipine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak area of nicardipine from the *Sample solution*

r_s = peak area of nicardipine from the *Standard solution*

C_s = concentration of USP Nicardipine Hydrochloride RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of nicardipine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• LIMIT OF N-BENZYL-N-METHYL-ETHANOLAMINE

Solution A: Dissolve 2.80 g of sodium perchlorate monohydrate in 1 L of water. Adjust with perchloric acid to a pH of 2.5.

Solution B: Acetonitrile and methanol (500:500)

Diluent: Acetonitrile and water (20:80)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
10	82	18
12	20	80
22	20	80
24	95	5
32	95	5

Standard solution: 2.5 μ g/mL of USP N-Benzyl-N-methyl-ethanolamine RS in *Diluent* prepared as follows. To a suitable amount of USP N-Benzyl-N-methyl-ethanolamine RS, add *Diluent* to 70% of the final volume. Sonicate to dissolve. Cool, and dilute with *Diluent* to volume. Pass the solution through a suitable filter of 0.45- μ m pore size.

Sample solution: Nominally equivalent to 0.5 mg/mL of nicardipine hydrochloride in *Diluent* from a suitable volume of Injection. Pass the solution through a suitable filter of 0.45- μ m pore size.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1.5 mL/min

Temperatures

Column: 30°

Sample: 10°

Injection volume: 50 μL

System suitabilitySample: *Standard solution***Suitability requirements**

Tailing factor: NMT 2.0

Relative standard deviation: NMT 5.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of *N*-benzyl-*N*-methyl-ethanolamine in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of *N*-benzyl-*N*-methyl-ethanolamine in the *Sample solution* r_S = peak response of *N*-benzyl-*N*-methyl-ethanolamine in the *Standard solution* C_S = concentration of USP *N*-Benzyl-*N*-methyl-ethanolamine RS in the *Standard solution* (mg/mL) C_U = nominal concentration of nicardipine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.7%

• **ORGANIC IMPURITIES****Solution A:** 3.5 g/L of sodium perchlorate monohydrate in water. Add 1 mL/L of triethylamine, and adjust with perchloric acid to a pH of 2.0.**Solution B:** Acetonitrile and methanol (700:300)Mobile phase: See *Table 2*.**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	70	30
15	70	30
55	35	65
60	35	65
62	70	30
70	70	30

Standard solution: 0.02 mg/mL of USP Nicardipine Hydrochloride RS in methanol prepared as follows. To a suitable amount of USP Nicardipine Hydrochloride RS add methanol to 60% of the final volume. Sonicate to dissolve. Cool, and dilute with methanol to volume. Pass the solution through a suitable filter of 0.45-μm pore size.**Sample solution:** Nominally equivalent to 2 mg/mL of nicardipine hydrochloride in methanol from a suitable volume of Injection. Pass the solution through a suitable filter of 0.45-μm pore size. [NOTE—*Sample solution* is stable for about 42 h at 10°.]**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Temperatures

Column: 50°

Sample: 10°

Injection volume: 10 μL

System suitabilitySample: *Standard solution***Suitability requirements**

Tailing factor: NMT 2.0

Relative standard deviation: NMT 5.0%

AnalysisSamples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 1/F \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_S = peak response of nicardipine from the *Standard solution* C_S = concentration of nicardipine hydrochloride in the *Standard solution* (mg/mL) C_U = nominal concentration of nicardipine hydrochloride in the *Sample solution* (mg/mL) F = relative response factor (see *Table 3*)Acceptance criteria: See *Table 3*.**Table 3**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Nicardipine monoacid ^a	0.72	1.00	0.2
Nicardipinepyridine analog ^b	0.94	0.42	0.9
Nicardipine	1.00	1.00	—
Any unspecified degradation impurity	—	—	0.2
Total impurities ^c	—	—	3.5

^a 5-(Methoxycarbonyl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid.^b 3-[2-[Benzyl(methyl)amino]ethyl] 5-methyl 2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate.^c Total impurities include the sum of all organic impurities and *N*-benzyl-*N*-methyl-ethanolamine.**OTHER COMPONENTS**• **CONTENT OF SORBITOL****Buffer:** 1 g/L of tetrabutylammonium hydrogen sulfate in water**Mobile phase:** Acetonitrile and *Buffer* (700:300)**Standard solution:** 4.8 mg/mL of USP Sorbitol RS in *Mobile phase*. Pass the solution through a suitable filter of 0.45-μm pore size. Sonication may be necessary to aid in dissolution.**Sample solution:** Nominally equivalent to 4.8 mg/mL of sorbitol in *Mobile phase* from the contents of NLT 3 injection vials. Pass the solution through a suitable filter of 0.45-μm pore size. [NOTE—*Sample solution* is stable for about 24 h.]

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 4.6-mm × 25-cm; 5-μm packing L8

Flow rate: 1 mL/min

Temperatures

Column: 40°

Detector: 50°

Injection volume: 25 μL

Run time: NLT 2 times the retention time of sorbitol

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of sorbitol in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of sorbitol from the *Sample solution*
 r_S = peak response of sorbitol from the *Standard solution*
 C_S = concentration of sorbitol in the *Standard solution* (mg/mL)

 C_U = nominal concentration of sorbitol in the *Sample solution* (mg/mL)

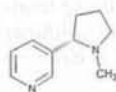
Acceptance criteria 90.0%–110.0%

SPECIFIC TESTS

- BACTERIAL ENDOTOXINS TEST** (85): NMT 8.33 USP Endotoxin Units/mg of nicardipine hydrochloride
- STERILITY TESTS** (71): Meets the requirements
- PH** (791): 3.0–3.9
- PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections
- OTHER REQUIREMENTS**: Meets the requirements for *Injections and Implanted Drug Products* (1)

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE**: Preserve in single-dose amber glass vials.
- LABELING**: Label it to indicate that it is to be diluted to the appropriate strength with a suitable intravenous fluid prior to administration.
- USP REFERENCE STANDARDS** (11)
 - USP *N*-Benzyl-*N*-methyl-ethanolamine RS
 - 2-[Benzyl(methyl)amino]ethanol.
 $C_{10}H_{15}NO$ 165.23
 - USP Endotoxin RS
 - USP Nicardipine Hydrochloride RS
 - USP Sorbitol RS
 - D-Glucitol;
(2*S*,3*R*,4*R*,5*R*)-Hexane-1,2,3,4,5,6-hexol.
 $C_6H_{14}O_6$ 182.17

Nicotine

$C_{10}H_{14}N_2$
3-(1-Methyl-2-pyrrolidinyl)pyridine;
β-Pyridyl-α-*N*-methyl pyrrolidine [54-11-5].

162.23

DEFINITIONNicotine contains NLT 99.0% and NMT 101.0% of nicotine ($C_{10}H_{14}N_2$), calculated on the anhydrous basis.**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197F)

Standard: Transfer an amount of USP Nicotine Bitartrate Dihydrate RS equivalent to 100 mg of nicotine to a 100-mL glass-stoppered tube. Add 20 mL of 1 M ammonium hydroxide, 5 mL of 10 M sodium hydroxide, and 20 mL of *n*-hexane. Shake for 5 min, and allow the phases to separate. Transfer the upper hexane phase to an evaporating dish, and evaporate on a steam bath.

Sample: Nicotine**Acceptance criteria**: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY• **PROCEDURE**

Sample solution: Dissolve 60 mg of Nicotine in 40 mL of glacial acetic acid.

Blank: 40 mL of glacial acetic acid**Titrimetric system**(see *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS**Endpoint detection**: Potentiometric**Analysis****Samples**: *Sample solution* and *Blank*Titrate the *Sample solution* with *Titrant*.Calculate the percentage of nicotine ($C_{10}H_{14}N_2$) in the portion of Nicotine taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F/W] \times 100$$

 V_S = *Titrant volume consumed by the Sample solution* (mL)

 V_B = *Titrant volume consumed by the Blank* (mL)

 N = *normality of the Titrant* (mEq/mL)

 F = *equivalency factor*, 81.1 mg/mEq

 W = *Sample weight* (mg)
Acceptance criteria: 99.0%–101.0% on the anhydrous basis**IMPURITIES****Delete the following:**

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm (Official 1-

Jan-2018)

• **ORGANIC IMPURITIES**

Solution A: Add 25 mL of 1 M acetic acid to 900 mL of water, then add 6 mL of ammonium hydroxide. Adjust with either 2 M acetic acid or 2 M ammonium hydroxide to a pH of 10.0, and dilute with water to 1000 mL.

Solution B: Acetonitrile**Mobile phase**: See *Table 1*.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
3.01	95	5
28	74	26
32	60	40

System suitability solution: 1.5 mg/mL of USP Nicotine Bitartrate Dihydrate RS and 6 μg/mL each of USP Nicotine Related Compound A RS, USP Nicotine Related Compound B RS, USP Nicotine Related Compound C

RS, USP Nicotine Related Compound D RS, USP Nicotine Related Compound E RS, USP Nicotine Related Compound F RS, and USP Nicotine Related Compound G RS in water. [NOTE—The concentration of each related compound is in terms of the free base.]

Standard solution: 2.5 µg/mL of USP Nicotine Bitartrate Dihydrate RS in water

Sensitivity solution: 1.2 µg/mL of USP Nicotine Bitartrate Dihydrate RS in water from the *Standard solution*

Sample solution: 0.8 mg/mL of Nicotine in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.5 between nicotine and nicotine related compound G, *System suitability solution*

Tailing factor: NMT 2.0 for nicotine, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Nicotine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of nicotine from the *Standard solution*

C_S = concentration of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis in the *Standard solution* (mg/mL)

C_U = concentration of Nicotine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of nicotine, 162.23

M_{r2} = molecular weight of anhydrous nicotine bitartrate, 462.41

Acceptance criteria: See *Table 2*. Disregard peaks that are less than 0.05% of the nicotine peak.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nicotine related compound E	0.3	0.3
Nicotine related compound C	0.55	0.3
Nicotine related compound F	0.7	0.3
Nicotine related compound A	0.8	0.3
Nicotine related compound D	0.86	0.3
Nicotine related compound G	0.9	0.3
Nicotine	1.00	—
Nicotine related compound B	1.6	0.3
Any other unspecified impurity	—	0.10
Total impurities	—	0.8

SPECIFIC TESTS

• OPTICAL ROTATION, *Specific Rotation* (781S)

Sample solution: 20 mg/mL in alcohol

Acceptance criteria: −130° to −143°

• WATER DETERMINATION, *Method I* (921): NMT 0.5%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Store under nitrogen in well-closed containers below 25°, protected from light and moisture.

• USP REFERENCE STANDARDS (11)

USP Nicotine Bitartrate Dihydrate RS

USP Nicotine Related Compound A RS

Anatabine;

1,2,3,6-Tetrahydro-2,3'-bipyridine.

$C_{10}H_{12}N_2$ 160.22

USP Nicotine Related Compound B RS

Nicotyrine;

3-(1-Methyl-1H-pyrrol-2-yl)pyridine.

$C_{10}H_{10}N_2$ 158.20

USP Nicotine Related Compound C RS

Cotinine;

(S)-1-Methyl-5-(pyridin-3-yl)pyrrolidin-2-one.

$C_{10}H_{12}N_2O$ 176.22

USP Nicotine Related Compound D RS

Myosmine;

3-(4,5-Dihydro-3H-pyrrol-2-yl)pyridine fumarate.

$C_9H_{10}N_2 \cdot C_4H_4O_4$ 262.26

USP Nicotine Related Compound E RS

Nicotine N-oxide;

(1R,2S)-1-Methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide oxalate.

$C_{10}H_{14}N_2O \cdot C_2H_2O_4$ 268.27

USP Nicotine Related Compound F RS

Nornicotine;

3-(Pyrrolidin-2-yl)pyridine.

$C_9H_{12}N_2$ 148.20

USP Nicotine Related Compound G RS

Anabasine;

(S)-3-(Piperidin-2-yl)pyridine.

$C_{10}H_{14}N_2$ 162.23

Nicotine Transdermal System

» Nicotine Transdermal System contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nicotine ($C_{10}H_{14}N_2$).

Packaging and storage—Preserve in the hermetic, light-resistant, unit-dose pouch.

Labeling—The labeling indicates the *Drug Release Test* with which the product complies.

USP Reference standards (11)—

USP Nicotine Bitartrate Dihydrate RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Drug release (724)—

TEST 1—If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 1*.

Medium: Phosphoric acid solution (1 in 1000); 250 mL, in a tall-form beaker.

Apparatus 7—Proceed as directed in the chapter, using the transdermal system holder-cylinder (see *Figure 4b*). Center the Transdermal System onto a dry, unused 10-cm × 10-cm piece of Cuprophane dialysis membrane with the adhesive side against the membrane, taking care to eliminate

air bubbles between the membrane and the release surface. Attach the membrane to the cylinder using two Parker O-rings, such that one of the borders of the transdermal system is aligned to the groove and it is wrapped around the cylinder. The filled beakers are weighed and pre-equilibrated to $32.0 \pm 0.3^\circ$, prior to immersing the test sample. Reciprocate at a frequency of about 30 cycles per minute with an amplitude of 2.0 ± 0.1 cm. At the end of each time interval, transfer the test sample to a fresh beaker containing the appropriate volume of *Medium*, weighed and pre-equilibrated to $32.0 \pm 0.3^\circ$. At the end of each release interval, allow the beakers to cool to room temperature, make up for evaporative losses by adding water to obtain the original weight, and mix. This solution is the final *Test solution*.

Times: 2, 12, and 24 hours.

Determine the amount of $C_{10}H_{14}N_2$ released by employing the following method.

Mobile phase—Transfer 0.2 mL of *N,N*-dimethyloctylamine to a 1-L volumetric flask, add 220 mL of acetonitrile, and mix. Add 300 mL of water, 0.2 mL of glacial acetic acid, 0.20 g of anhydrous sodium acetate, and 0.55 g of sodium 1-dodecanesulfonate, and dilute with water to volume. Mix for 1 hour until clear. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—Equilibration of the column may take as long as 3 hours.]

Standard solution—Dissolve an accurately weighed quantity of USP Nicotine Bitartrate Dihydrate RS in *Medium*, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration of about 0.142 mg of nicotine bitartrate per mL (or 0.046 mg nicotine as free base per mL). [NOTE—About 80 mL of this solution is required in order to prepare the *System suitability solution*.]

System suitability solution—Transfer 8 mg (free base) of nicotine to a 100-mL volumetric flask, and dissolve in 10 mL of acetonitrile. Add 5 mL of 30% hydrogen peroxide, and allow 15 minutes to react. Dilute with *Medium* to volume, and mix. Transfer 20 mL of this solution to a 100-mL volumetric flask, dilute with *Standard solution* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm \times 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between nicotine and any degradation peaks is not less than 1.1; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 50 μ L) of filtered portions of the *Standard solution* and the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

Tolerances—The amount of $C_{10}H_{14}N_2$ released, as a percentage of the labeled amount of the dose absorbed in vivo, at the times specified below, conforms to *Acceptance Table 1*.

Time (hours)	Amount dissolved
0–2	between 31% and 87%
2–12	between 62% and 191%
12–24	between 85% and 261%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 2*.

Phosphate buffer—Dissolve 40.0 g of sodium chloride, 1.0 g of potassium chloride, 8.66 g of dibasic sodium phosphate, and 1.0 g of monobasic potassium phosphate in 5 L of water.

Medium: Phosphate buffer; 500 mL.

Apparatus 6: 50 rpm, double-sided tape being used to attach the Transdermal System to the cylinder.

Times: 6 and 24 hours.

Determine the amount of $C_{10}H_{14}N_2$ released by employing the following method.

Mobile phase—Proceed as directed in the *Assay*.

System suitability solution—Transfer 1.0 mL of the *System suitability solution*, prepared as directed in the *Assay*, to a 100-mL volumetric flask, dilute with *Medium* to volume, and mix.

Standard solution—Pipet 6.0 mL of the *Standard preparation*, prepared as directed in the *Assay*, into a 50-mL volumetric flask, dilute with *Medium* to volume, and mix. Dilute quantitatively and stepwise with *Medium* to obtain an appropriate final concentration.

Test solution—At each of the test times, withdraw a 2-mL aliquot of the solution under test. [NOTE—Replace the aliquots withdrawn for analysis with fresh portions of *Medium*.]

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 260-nm detector and a 4.6-mm \times 12.5-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution* used for the 6-hour interval, and record the peak responses as directed for *Procedure*: the resolution, *R*, between 4,4'-dipyridyl and nicotine is not less than 5.0; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 100 μ L) of the filtered portion of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

Tolerances—The amount of $C_{10}H_{14}N_2$ released, as a percentage of the labeled amount of the dose absorbed in vivo, at the times specified, conforms to *Acceptance Table 1*.

Time (hours)	Amount dissolved
6	between 71% and 157%
24	between 156% and 224%

TEST 3—If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 3*.

Medium: water; 900 mL.

Apparatus 5: 50 rpm, the stainless steel disk assembly being replaced with a 5-cm watch glass for an 11-mg Transdermal System and an 8-cm watch glass for a 22-mg Transdermal System.

Times: 1, 2, and 4 hours.

Standard solution—Prepare a solution of USP Nicotine Bitartrate Dihydrate RS in water having a known concentration of nicotine similar to that of the solution under test.

Procedure—Determine the amount of $C_{10}H_{14}N_2$ released by employing UV absorption at the wavelength of maximum absorbance at about 259 nm, in comparison with the *Standard solution*, using water as the blank.

Tolerances—The amount of $C_{10}H_{14}N_2$ released, as a percentage of the labeled amount of the dose absorbed in vivo, at the times specified, conforms to the following *Acceptance Table*.

Time (hours)	Amount dissolved
1	between 35% and 75%
2	between 55% and 95%
4	not less than 73%

Acceptance Table

Level	Tested	Criteria
L ₁	6	No individual value lies outside each of the stated ranges and no individual value is less than the stated amount at the final test time.
L ₂	6	The average value of the 12 units (L ₁ + L ₂) lies within each of the stated ranges and is not less than the stated amount at the final test time; none is more than 5% of the labeled content outside each of the stated ranges; and none is more than 5% of the labeled content below the stated amount at the final test time.
L ₃	12	The average value of the 24 units (L ₁ + L ₂ + L ₃) lies within each of the stated ranges and is not less than the stated amount at the final test time; not more than 2 of the 24 units are more than 5% of labeled content outside each of the stated ranges; not more than 2 of the 24 units are more than 5% of the labeled content below the stated amount at the final test time; and none of the units is more than 10% of the labeled content outside each of the stated ranges or more than 10% of the labeled content below the stated amount at the final test time.

TEST 4—If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 4*.

Medium: 0.025 N hydrochloric acid; 600 mL.

Apparatus 5: 50 rpm, a convex screen being used to hold the Transdermal System in position during testing.

Times: 4 and 16 hours.

Standard solution and Procedure—Proceed as directed under Test 3.

Tolerances—The amount of C₁₀H₁₄N₂ released, as a percentage of the labeled amount of the dose absorbed in vivo, at the times specified, conforms to Acceptance Table 1.

Time (hours)	Amount dissolved
4	between 36% and 66%
16	between 72% and 112%

TEST 5—If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 5*.

Phosphate buffer, Medium, and Apparatus—Proceed as directed under Test 2.

Times: 3, 6, and 24 hours.

Mobile phase—Proceed as directed in the Assay.

System suitability solution, Standard solution, Test solution, and Chromatographic system—Proceed as directed under Test 2.

Procedure—Proceed as directed under Test 2 except to inject about 30 μ L.

Tolerances—The amount of C₁₀H₁₄N₂ released, as a percentage of the labeled amount of the dose absorbed in vivo, at the times specified, conforms to Acceptance Table 1.

Time (hours)	Amount dissolved
3	between 79% and 112%
6	between 108% and 141%
24	between 156% and 202%

Uniformity of dosage units (905): meets the requirements.

Assay—

Mobile phase—Mix 300 mL of acetonitrile, 700 mL of water, and 1 mL of triethylamine, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Nicotine Bitartrate Dihydrate RS in water to obtain a stock solution having a known concentration of about 26.87 mg per mL. Quantitatively dilute a volume of the stock solution with methanol to obtain a solution having a known concentration of about 5.37 mg of USP Nicotine Bitartrate Dihydrate RS per mL. [NOTE—This solution contains 1.75 mg of nicotine per mL.]

System suitability solution—Transfer about 8 mg of 4,4'-dipyridyl to a 25-mL volumetric flask, add 5.0 mL of the *Standard preparation*, dilute with methanol to volume, and mix.

Assay preparation—Cut an accurately counted number of Transdermal Systems, equivalent to about 175 mg of nicotine, based on the label claim, into strips 5 cm² in area. Remove the protective liners, if any, from the strips, and discard. Transfer the strips to a 250-mL flask, and add 100.0 mL of methanol. Insert the stopper into the flask, and shake by mechanical means for about 3 hours. Filter, and use the clear filtrate as the *Assay preparation*.

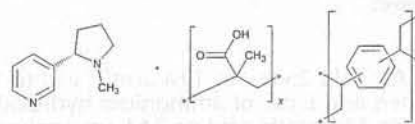
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 260-nm detector and a 4.6-mm \times 25-cm column containing packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between nicotine and 4,4'-dipyridyl is not less than 5.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percent label claim of nicotine (C₁₀H₁₄N₂) in each Transdermal System taken by the formula:

$$100(162.23/462.41)(C_S / C_U)(r_U / r_S)$$

in which 162.23 and 462.41 are the molecular weights of nicotine and anhydrous nicotine bitartrate, respectively; *C_S* is the concentration, in mg per mL, of USP Nicotine Bitartrate Dihydrate RS in the *Standard preparation*; *C_U* is the nominal concentration of nicotine in the *Assay preparation*, based on the label claim; and *r_U* and *r_S* are the nicotine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nicotine Polacrilex



[(C₄H₆O₂)_x(C₁₀H₁₀)_y](C₁₀H₁₄N₂)

2-Propenoic acid, 2-methyl-, polymer with diethenylbenzene, complex with (S)-3-(1-methyl-2-pyrrolidinyl)pyridine;

Methacrylic acid polymer with divinylbenzene, complex with nicotine [96055-45-7].

DEFINITION

Change to read:

Nicotine Polacrilex is a weak carboxylic cation-exchange resin prepared from methacrylic acid and divinylbenzene, in complex with nicotine. It contains NLT 95.0% and NMT 115.0% of the labeled amount of nicotine ($C_{10}H_{14}N_2$), calculated on the Δ dried Δ_{USP40} basis.

Δ [NOTE—Nicotine Polacrilex is also known as Nicotine Resinate.] Δ_{USP40}

IDENTIFICATION

Change to read:

- **A. INFRARED ABSORPTION** Δ (197) **FOR NICOTINE:** [NOTE—Methods described in (197K) or (197A) may be used.] Δ_{USP40}

Sample: Transfer an amount of Nicotine Polacrilex equivalent to 100 mg of nicotine to a 100-mL glass-stoppered tube. Add 20 mL of 1 M ammonium hydroxide, 5 mL of 10 M sodium hydroxide, and 20 mL of *n*-hexane. Shake for 5 min, and allow the phases to separate. Transfer the upper hexane phase to an evaporating dish, and evaporate on a steam bath.

Standard: Use USP Nicotine Bitartrate Dihydrate RS, and prepare as directed for the *Sample*.

Acceptance criteria: Meets the requirements

Change to read:

- **B. INFRARED ABSORPTION** Δ (197) **FOR POLACRILEX:** [NOTE—Methods described in (197K) or (197A) may be used.] Δ_{USP40}

Standard: Transfer a portion of USP Polacrilex Resin RS, equivalent to the amount of Nicotine Polacrilex used to prepare the *Sample solution* in the *Assay*, to a glass-stoppered tube. Add 10 mL of 1 M ammonium hydroxide, shake for 10 min, then centrifuge. Decant the ammonia solution from the residue, and wash the residue by shaking it with three 10-mL volumes of water, decanting the water phase after each shaking. Wash with 10 mL of 0.1 N hydrochloric acid, decant the liquid, and dry the residue at 105°.

Sample: Use the residue obtained from the *Sample solution* in the *Assay*. Decant the ammonia solution remaining from the residue, and wash the residue by shaking it with three 10-mL volumes of water, decanting the water phase after each shaking. Wash with 10 mL of 0.1 N hydrochloric acid, decant the liquid, and dry the residue at 105°.

Acceptance criteria: Meets the requirements

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• PROCEDURE

Solution A: Add 25 mL of 1 M acetic acid to 900 mL of water, then add 6 mL of ammonium hydroxide. Adjust with either 2 M acetic acid or 2 M ammonium hydroxide to a pH of 10.0, and dilute with water to 1000 mL.

Solution B: Acetonitrile

Mobile phase: See Table 1. Δ [NOTE—Re-equilibration time may be adjusted, if necessary.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
3.01	95	5
28	74	26
32	60	40
33	100	0
35	100	0

Δ_{USP40}

System suitability solution: 1.5 mg/mL of USP Nicotine Bitartrate Dihydrate RS and 6 μ g/mL of USP Nicotine Related Compound G RS in water

Standard solution: 1.8 mg/mL of USP Nicotine Bitartrate Dihydrate RS in water

Sample solution: Nominally 0.6 mg/mL of nicotine prepared as follows. Transfer an amount of Nicotine Polacrilex equivalent to 30 mg of nicotine to a glass-stoppered tube. Add 10.0 mL of 1 M ammonium hydroxide, shake vigorously for 10 min, then centrifuge. Transfer 5.0 mL of the clear solution to a 25-mL volumetric flask, add 5 mL of 1 M acetic acid, and dilute with water to volume. Retain the residue from centrifugation for use in *Identification B*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for the relative retention times.]

Suitability requirements

Resolution: NLT 2.5 between nicotine and nicotine related compound G, *System suitability solution*

Tailing factor: NMT 2.0 for nicotine, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nicotine ($C_{10}H_{14}N_2$) in the portion of Nicotine Polacrilex taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of nicotine from the *Sample solution*

r_S = peak response of nicotine from the *Standard solution*

C_S = concentration of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis in the *Standard solution* (mg/mL)

C_U = nominal concentration of nicotine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of nicotine, 162.23

M_{r2} = molecular weight of anhydrous nicotine bitartrate, 462.41

Acceptance criteria: 95.0%–115.0% on the Δ dried Δ_{USP40} basis

IMPURITIES

• ORGANIC IMPURITIES

Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 1.5 mg/mL of USP Nicotine Bitartrate Dihydrate RS and 6 μ g/mL each of USP

Nicotine Related Compound A RS, USP Nicotine Related Compound B RS, USP Nicotine Related Compound C RS, USP Nicotine Related Compound D RS, USP Nicotine Related Compound E RS, USP Nicotine Related Compound F RS, and USP Nicotine Related Compound G RS in water. [NOTE—The concentration of each related compound is in terms of the free base.]

Standard solution: 1.8 µg/mL of USP Nicotine Bitartrate Dihydrate RS in water

Sensitivity solution: 0.9 µg/mL of USP Nicotine Bitartrate Dihydrate RS in water from the *Standard solution*

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—See Table 2 for the relative retention times.]

Suitability requirements

Resolution: NLT 2.5 between nicotine and nicotine related compound G, *System suitability solution*

Tailing factor: NMT 2.0 for nicotine, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Nicotine Polacrilex taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of nicotine from the *Standard solution*

C_S = concentration of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis in the *Standard solution* (mg/mL)

C_U = nominal concentration of nicotine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of nicotine, 162.23

M_{r2} = molecular weight of anhydrous nicotine bitartrate, 462.41

Acceptance criteria: See Table 2. Disregard peaks that are less than 0.05% of the nicotine peak.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nicotine related compound E	0.3	0.3
Nicotine related compound C	0.55	0.3
Nicotine related compound F	0.7	0.3
Nicotine related compound A	0.8	0.3
Nicotine related compound D	0.86	0.3
Nicotine related compound G	0.9	0.3
Nicotine	1.00	—
Nicotine related compound B	1.6	0.3
Any other unspecified impurity	—	0.10
Total impurities	—	0.8

SPECIFIC TESTS

Delete the following:

▲ WATER DETERMINATION, Method I (921)

Sample solution: Transfer about 1.0 g of Nicotine Polacrilex to a 50-mL glass-stoppered test tube, and add 20.0 mL of methanol. Shake for 30 min, and allow to stand for 30 min. Use a 10-mL portion of the methanol layer for the titration.

Acceptance criteria: NMT 5.0% ▲USP40

Add the following:

▲ LOSS ON DRYING (731)

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 7.0% ▲USP40

Change to read:

• NICOTINE RELEASE

Solution A: 9 mg/mL of sodium chloride in water

Sample stock solution: Transfer an amount of Nicotine Polacrilex equivalent to 4 mg of nicotine to a glass-stoppered tube, add 10.0 mL of *Solution A* that has been warmed to 37°, and shake by mechanical means for 10 min. Immediately pass the liquid through a dry filter paper, discarding the first mL of the filtrate.

Sample solution: Transfer 1.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.

Instrumental conditions

▲(See *Ultraviolet-Visible Spectroscopy* (857).) ▲USP40

Mode: UV

Analytical wavelengths: 236, 259, and 282 nm

Blank: 1.0 mL of *Solution A* diluted with 0.1 N hydrochloric acid to 25 mL

Analysis

Samples: *Sample solution* and *Blank*

Calculate the percentage of nicotine released:

$$\text{Result} = (A_{259} - 0.5A_{236} - 0.5A_{282}) \times (V/E) \times (F/W) \times (1/P) \times 100$$

A_{259} = absorbance of the *Sample solution*, corrected for the *Blank* absorbance, at a wavelength of 259 nm

A_{236} = absorbance of the *Sample solution*, corrected for the *Blank* absorbance, at a wavelength of 236 nm

A_{282} = absorbance of the *Sample solution*, corrected for the *Blank* absorbance, at a wavelength of 282 nm

V = dilution volume, 250 mL

E = specific absorbance of nicotine at a wavelength of 259 nm, 323 ▲mL g⁻¹ cm⁻¹

▲USP40

F = unit conversion factor, 1000 mg/g

W = weight of Nicotine Polacrilex (mg)

P = percentage of nicotine in Nicotine Polacrilex determined in the *Assay*

Acceptance criteria: NLT 70% in 10 min

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers, ▲protected from light. ▲USP40

Change to read:

• USP REFERENCE STANDARDS (11)

USP Nicotine Bitartrate Dihydrate RS

USP Nicotine Related Compound A RS

Anatabine;

1,2,3,6-Tetrahydro-2,3'-bipyridine.

C₁₀H₁₂N₂ 160.22

USP Nicotine Related Compound B RS

Nicotyrine;

3-(1-Methyl-1H-pyrrol-2-yl)pyridine.

C₁₀H₁₀N₂ 158.20

USP Nicotine Related Compound C RS
Cotinine;
(S)-1-Methyl-5-(pyridin-3-yl)pyrrolidin-2-one.
 $C_{10}H_{12}N_2O$ 176.22

USP Nicotine Related Compound D RS
Myosmine;
3-(4,5-Dihydro-3H-pyrrol-2-yl)pyridine fumarate.
 $C_9H_{10}N_2 \cdot C_4H_4O_4$ 262.26

USP Nicotine Related Compound E RS
Nicotine N-oxide;
(1R,2S)-1-Methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide oxalate.
 $C_{10}H_{14}N_2O \cdot C_2H_2O_4$ 268.27

USP Nicotine Related Compound F RS
Nornicotine;
3-(Pyrrolidin-2-yl)pyridine.
 $C_9H_{12}N_2$ 148.20

USP Nicotine Related Compound G RS
Anabasine;
(S)-3-(Piperidin-2-yl)pyridine.
 $C_{10}H_{14}N_2$ 162.23

• USP Polacrilex Resin RS (ERR 1-Jun-2016)

Nicotine Polacrilex Gum

» Nicotine Polacrilex Gum contains an amount of Nicotine Polacrilex $[(C_4H_6O_2)_x(C_{10}H_{10})_y](C_{10}H_{14}N_2)$ equivalent to not less than 90 percent and not more than 120 percent of the labeled amount of nicotine ($C_{10}H_{14}N_2$).

USP Reference standards (11)—

USP Nicotine Bitartrate Dihydrate RS
USP Polacrilex Resin RS

Identification—

A: Developing solvent—Prepare a mixture of chloroform, acetone, and diethylamine (40:5:5).

Test solution—Cut several pieces of Gum into small pieces with scissors, and weigh. Transfer a portion of the Gum, equivalent to about 4 mg of nicotine, to a centrifuge tube. Add 5 mL of chloroform, sonicate for about 30 minutes to dissolve the nicotine, and centrifuge for about 10 minutes. Cool to about 15°, and add two 3-mL portions of 0.5 N hydrochloric acid with gentle mixing, and release excess pressure if necessary. Mix the contents of the tube by shaking, and centrifuge the mixture for about 10 minutes. Transfer 5 mL of the upper aqueous layer to a separatory funnel, and adjust with 0.5 N sodium hydroxide solution to a pH greater than 10.0. Add 3 mL of chloroform, and shake gently. Use the chloroform layer as the *Test solution*.

Standard solution—Transfer 10 mg of USP Nicotine Bitartrate Dihydrate RS to a separatory funnel, add 10 mL of water, and mix to dissolve. Adjust with 0.5 N sodium hydroxide to a pH greater than 10.0. Add 3 mL of chloroform, with gentle shaking, and use the chloroform layer as the *Standard solution*.

Procedure—Separately apply 10 μ L each of the *Test solution* and the *Standard solution* about 1.5 cm from the lower edge of a thin-layer chromatographic plate (see *Chromatography* (621)). Air-dry, place the plate in a chromatographic tank that has been saturated with *Developing solvent*, and develop the chromatogram until the solvent front has moved about 7 cm. Remove the plate from the chamber, and allow to air-dry. Examine the plate under short-wave-length UV light: the R_f value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution* (presence of nicotine).

B: Infrared Absorption (197K)—Use USP Polacrilex Resin RS and a test specimen prepared as follows. Cut a piece of

Gum into small pieces with scissors. Place the pieces in a 50-mL centrifuge tube, add about 20 mL of *n*-hexane, and place in an ultrasonic bath for about 30 minutes. Centrifuge at about 2500 rpm for about 5 minutes. Decant the hexane phase, and add 10 mL of 2 N hydrochloric acid. Shake the tube carefully, and open the stopper slightly to relieve any excess pressure. Add 10 mL of alcohol, and shake the tube carefully with the stopper slightly open. Centrifuge again as described above, and decant the liquid, taking care to avoid contamination of the precipitate with the gum material. Add 1 mL to 3 mL of water, mix gently to resuspend the precipitate, and filter. Wash the residue on the filter with water and then with alcohol. Dry the filter and residue at about 105° for 1 hour (presence of polacrilex).

Uniformity of dosage units (905): meets the requirements.

Assay—

Acetate buffer—Prepare a mixture containing 13.6 g of sodium acetate and 57.2 mL of glacial acetic acid in 1000 mL of water.

Solvent—Prepare a mixture of water, acetonitrile, 0.25 M sodium 1-decanesulfonate, and *Acetate buffer* (785:150:40:25).

Mobile phase—Prepare a mixture containing water, acetonitrile, *Acetate buffer*, and 0.25 M sodium 1-decanesulfonate (685:200:75:40).

Standard preparation—Dissolve an accurately weighed quantity of USP Nicotine Bitartrate Dihydrate RS in *Solvent* to obtain a *Standard stock solution* having a known concentration of about 1.25 mg per mL. Dilute a volume of this solution quantitatively with *Solvent* to obtain a *Standard preparation* having a known concentration of about 125 μ g per mL (40 μ g of nicotine per mL).

Assay preparation—Place one piece of Gum, accurately weighed, in a stoppered flask, add about 50 mL of *n*-hexane, and transfer 50.0 mL of *Solvent*. Add a stirring bar, insert the stopper in the flask, and stir vigorously for about 30 minutes or until the test specimen has been dispersed. Remove from the stirring mechanism, and allow to stand for about 30 minutes or until the phases have separated. Remove an aliquot of the lower layer, taking care not to remove a large quantity of the insoluble excipients, and filter, discarding the first few mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

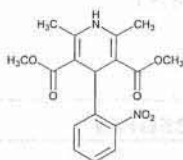
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm \times 30-cm stainless steel column containing packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, record the chromatograms, and measure the peak responses as directed for *Procedure*: the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Perform the following procedure on 10 individual pieces of Gum, and use the average of the calculated values as the assay value. Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 50 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of nicotine ($C_{10}H_{14}N_2$) in the Gum taken by the formula:

$$50C(162.23 / 462.41)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Nicotine Bitartrate Dihydrate RS on the anhydrous basis in the *Standard preparation*, 162.23 and 462.41 are the molecular weights of nicotine and anhydrous nicotine bitartrate, respectively, and r_U and r_S are peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nifedipine



$C_{17}H_{18}N_2O_6$ 346.33
3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester;
Dimethyl 1,4-dihydro-2,6-dimethyl-4-(o-nitrophenyl)-3,5-pyridinedicarboxylate [21829-25-4].

DEFINITION

Nifedipine contains NLT 98.0% and NMT 102.0% of nifedipine ($C_{17}H_{18}N_2O_6$), calculated on the dried basis. [NOTE—Nifedipine, when exposed to daylight and certain wavelengths of artificial light, readily converts to a nitrosophenylpyridine derivative. Exposure to UV light leads to the formation of a nitrophenylpyridine derivative. Perform the Assay and other tests in the dark or under golden fluorescent or other low-actinic light. Use low-actinic glassware.]

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K): Do not dry samples.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Protect the *Standard solution* and the *Sample solution* from actinic light. Conduct the Assay promptly after preparation of the *Standard solution* and the *Sample solution*.

Mobile phase: Acetonitrile, methanol, and water (25:25:50)

Standard stock solution: 1 mg/mL of USP Nifedipine RS in methanol

Standard solution: 0.1 mg/mL in *Mobile phase* from *Standard stock solution*

Sample solution: 0.1 mg/mL of Nifedipine prepared as follows. Transfer about 25 mg of Nifedipine to a 250-mL flask, dissolve in 25 mL of methanol, and dilute with *Mobile phase* to volume.

Chromatographic system
(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 25 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 4000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nifedipine ($C_{17}H_{18}N_2O_6$) in the portion of Nifedipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Nifedipine RS in the *Standard solution* (mg/mL)

C_U = concentration of Nifedipine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%, an ignition temperature of 600° being used

Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 10 ppm (Official 1-Jan-2018)

ORGANIC IMPURITIES

Protect *Standard stock solution B* and the *Sample solution* from actinic light. Conduct this test promptly after preparation of *Standard stock solution B* and the *Sample solution*.

Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard stock solution A: 1 mg/mL of USP Nifedipine RS in methanol

Standard solution A: 0.3 mg/mL of USP Nifedipine RS in *Mobile phase* from *Standard stock solution A*

Standard stock solution B: 1 mg/mL of USP Nifedipine Nitrophenylpyridine Analog RS in methanol

Standard solution B: 0.6 μg/mL of USP Nifedipine Nitrophenylpyridine Analog RS in *Mobile phase* from *Standard stock solution B*

Standard stock solution C: 1 mg/mL of USP Nifedipine Nitrosophenylpyridine Analog RS in methanol

Standard solution C: 0.6 μg/mL of USP Nifedipine Nitrosophenylpyridine Analog RS from *Standard stock solution C*, diluted with *Mobile phase*

Standard solution D: *Mobile phase*, *Standard solution B*, and *Standard solution C* (1:1:1)

System suitability solution: *Standard solution A*, *Standard solution B*, and *Standard solution C* (1:1:1)

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog; NLT 1.0 between the nifedipine nitrosophenylpyridine analog and nifedipine

Relative standard deviation: NMT 10% for both nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog

Analysis

Samples: *Sample solution* and *Standard solution D*

Calculate the percentage of nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog in the portion of Nifedipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of nifedipine nitrophenylpyridine analog or nifedipine nitrosophenylpyridine analog from the *Sample solution*
 r_S = peak response of nifedipine nitrophenylpyridine analog or nifedipine nitrosophenylpyridine analog from *Standard solution D*
 C_S = concentration of USP Nifedipine Nitrophenylpyridine Analog RS or USP Nifedipine Nitrosophenylpyridine Analog RS in *Standard solution D* (mg/mL)
 C_U = concentration of Nifedipine in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nifedipine nitrophenylpyridine analog ^a	0.8	0.2
Nifedipine nitrosophenylpyridine analog ^b	0.9	0.2
Nifedipine	1.0	—

^a Dimethyl 4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.^b Dimethyl 4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.**• LIMIT OF CHLORIDE AND SULFATE**

Standard solution A: Add 5.0 mL of water to 10 mL of 8.2 µg/mL of sodium chloride (corresponding to 5 µg/mL of chloride).

Standard solution B: Equivalent to 10 µg/mL of sulfate from potassium sulfate in water.

Sample solution: To 5.0 g of Nifedipine add 4.0 mL of 6 N acetic acid and 46 mL of water. Bring carefully to a boil on a hot plate. Cool, pass through filter paper that is free of chloride and sulfate, and use the filtrate.

Chloride: Pipet 2.5 mL of the *Sample solution* into a 50-mL color-comparison tube, and add 12.5 mL of water. Into a matched color-comparison tube, pipet 10 mL of *Standard solution A*. To each tube add 0.15 mL of 0.3 M nitric acid and 0.3 mL of silver nitrate TS, and mix.

Acceptance criteria: NMT 0.02%; the opalescence exhibited by the *Sample solution* does not exceed that of *Standard solution A*.

Sulfate: Pipet into each of two 50-mL matched color-comparison tubes 1.5 mL of *Standard solution B*. To each tube add, successively and with continuous shaking, 0.75 mL of alcohol, 0.5 mL of a 6.1% aqueous solution of barium chloride, and 0.25 mL of 6 N acetic acid. Shake for an additional 30 s. Pipet into one tube, designated the standard tube, 15 mL of *Standard solution B*. Pipet into the other tube, designated the sample tube, 3 mL of *Sample solution* and 12 mL of water.

Acceptance criteria: NMT 0.05%; the turbidity exhibited by the *Sample solution* in the sample tube does not exceed that of *Standard solution B* in the standard tube.

• PERCHLORIC ACID TITRATION

Sample solution: Dissolve 4 g of Nifedipine in 160 mL of glacial acetic acid in a 250-mL conical flask with the aid of an ultrasonic bath.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint: Visual

Analysis: Add 3 drops of *p*-naphtholbenzein TS to the *Sample solution*, and titrate with *Titrant* to a green endpoint.

Acceptance criteria: NMT 0.12 mL of 0.1 N perchloric acid is consumed for each g of Nifedipine.

SPECIFIC TESTS**• LOSS ON DRYING (731)**

Analysis: Dry a sample at 105° to constant weight.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in tight, light-resistant containers.

• USP REFERENCE STANDARDS (11)

USP Nifedipine RS

USP Nifedipine Nitrophenylpyridine Analog RS

Dimethyl 4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.

C₁₇H₁₆N₂O₆ 344.33

USP Nifedipine Nitrosophenylpyridine Analog RS

Dimethyl 4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.

C₁₇H₁₆N₂O₅ 328.33

Nifedipine Capsules**DEFINITION**

Nifedipine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of nifedipine (C₁₇H₁₆N₂O₆).

Nifedipine, when exposed to daylight and certain wavelengths of artificial light, readily converts to a nitrosophenylpyridine derivative. Exposure to UV light leads to the formation of a nitrophenylpyridine derivative. Perform assays and tests in the dark or under golden fluorescent or other low-actinic light. Use low-actinic glassware.

IDENTIFICATION**• A.**

Standard solution: 1.2 mg/mL of USP Nifedipine RS in methylene chloride

Sample solution: Using the technique described in *Procedure for content uniformity* in the test for *Uniformity of Dosage Units* transfer the contents of 3 Capsules to a centrifuge tube, rinsing the scissors with 20 mL of 0.1 N sodium hydroxide. Pipet 25 mL of methylene chloride into the tube, insert a stopper, invert several times, and carefully release the pressure in the tube. Insert the stopper again tightly, and shake gently for 1 h. Centrifuge the tube for 10 min at 2000–2500 rpm. Remove the supernatant aqueous phase by aspiration with a syringe, and transfer 5.0 mL of the clarified lower layer to a suitable vial.

Mixed solution: Mixture of *Standard solution* and *Sample solution* (1:1)

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.5-mm layer of chromatographic silica gel mixture

Application volume: 500 µL

Developing solvent system: Ethyl acetate and cyclohexane (1:1)

Spray reagent stock solution: Dissolve 3 g of bismuth subnitrate and 30 g of potassium iodide, taken in a 100-mL volumetric flask, in 10 mL of 3 N hydrochloric acid, and dilute with water to volume.

Spray reagent: Before use, mix 10 mL of *Spray reagent stock solution* in a 100-mL volumetric flask with 10 mL of 3 N hydrochloric acid, and dilute with water to volume.

Analysis

Samples: *Standard solution*, *Sample solution*, and *Mixed solution*

Allow the spots to dry, and develop the chromatogram, protected from light, in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and air-dry the plate until no odor is detectable. Immediately view the plate under short-wavelength UV light, note the colored bands, and then spray the plate with *Spray reagent*.

Acceptance criteria: Each solution exhibits a dark blue major band at the same *R_f* value of 0.3 before the treatment with *Spray reagent*, and each solution exhibits a compact light orange band on a yellow background after the treatment with *Spray reagent*.

• B. The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE:** Protect the *Standard solution* and the *Sample solution* from actinic light. Conduct the Assay promptly after preparation of the *Standard solution* and the *Sample solution*.

Mobile phase: Acetonitrile, methanol, and water (25:25:50)

Standard stock solution: 1 mg/mL of USP Nifedipine RS in methanol

Standard solution: 0.1 mg/mL of USP Nifedipine RS in *Mobile phase* from *Standard stock solution*

Sample solution: 0.1 mg/mL of nifedipine prepared as follows. Transfer the contents of 5 Capsules with the aid of a small amount of methanol to a suitable volumetric flask, and dilute with *Mobile phase* to volume. Pass through a solvent-resistant filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Columns

Guard: Packing L1

Analytical: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 25 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 4000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nifedipine from the *Sample solution*

r_S = peak response of nifedipine from the *Standard solution*

C_S = concentration of USP Nifedipine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of nifedipine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)

Medium: Simulated gastric fluid TS (without pepsin); 900 mL

Apparatus 2: 50 rpm

Time: 20 min

Standard solution: Dissolve a quantity of USP Nifedipine RS in an amount of methanol not exceeding 2% of the final volume, and dilute with *Medium* to obtain a solution of a known suitable concentration.

Sample solution: Pass a portion of solution under test through a suitable filter, and dilute as needed with *Medium*, in comparison with the *Standard solution*. Filters must be checked for absorptive loss of nifedipine.

Instrumental conditions

Mode: UV

Analytical wavelength: 340 nm

Analysis

Samples: *Standard solution* and *Sample solution*
Determine the percentage of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved by using UV absorbances at the specified wavelength.

Tolerances: NLT 80% (Q) of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905)**Procedure for content uniformity**

Standard solution: 50 μg/mL of USP Nifedipine RS in methanol

Sample solution: With the point of a pair of sharp scissors, make a small hole at the end of 1 Capsule. Squeeze most of the contents into a 200-mL volumetric flask, cut the Capsule in half, and drop it into the flask. Rinse the scissors with 20 mL of methanol, quantitatively collecting the rinse in the flask. Dilute with methanol to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: 350 nm

Cell: 1 cm

Blank: Methanol

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Nifedipine RS in the *Standard solution* (μg/mL)

C_U = nominal concentration of nifedipine in the *Sample solution* (μg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES:** Protect the *Standard solution* and the *Sample solution* from actinic light. Conduct this test promptly after preparation of the *Standard solution* and the *Sample solution*.

Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard stock solution A: 1 mg/mL of USP Nifedipine RS in methanol

Standard solution A: 0.3 mg/mL of USP Nifedipine RS from *Standard stock solution A* in *Mobile phase*

Standard stock solution B: 1 mg/mL of USP Nifedipine Nitrophenylpyridine Analog RS in methanol

Standard solution B: 6 μg/mL of USP Nifedipine Nitrophenylpyridine Analog RS in *Mobile phase* from *Standard stock solution B*

Standard stock solution C: 1 mg/mL of USP Nifedipine Nitrosophenylpyridine Analog RS in methanol

Standard solution C: 1.5 μg/mL of USP Nifedipine Nitrosophenylpyridine Analog RS in *Mobile phase* from *Standard stock solution C*

Standard solution D: Mixture of *Standard solution B*, *Standard solution C*, and *Mobile phase* (1:1:1)

System suitability solution: Mixture of *Standard solution A*, *Standard solution B*, and *Standard solution C* (1:1:1)

Proceed as directed in the Assay.

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between the nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog peaks, and NLT 1.0 between the nifedipine nitrosophenylpyridine analog and nifedipine peaks

Relative standard deviation: NMT 10% for each nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog peaks

Analysis

Samples: *Standard solution D* and *Sample solution*
Calculate the percentage of nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of nifedipine nitrophenylpyridine analog or nifedipine nitrosophenylpyridine analog from the *Sample solution*
 r_S = peak response of nifedipine nitrophenylpyridine analog or nifedipine nitrosophenylpyridine analog from *Standard solution D*
 C_S = concentration of the appropriate USP Nifedipine Nitrophenylpyridine Analog RS or USP Nifedipine Nitrosophenylpyridine Analog RS in *Standard solution D* (mg/mL)
 C_U = nominal concentration of nifedipine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Nifedipine nitrophenyl analog ^a	0.8	2.0
Nifedipine nitrosophenyl analog ^b	0.9	0.5
Nifedipine	1.0	—

^a Dimethyl 4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.

^b Dimethyl 4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature between 15° and 25°.

- USP REFERENCE STANDARDS (11)**

USP Nifedipine RS

USP Nifedipine Nitrophenylpyridine Analog RS

Dimethyl 4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.

$C_{17}H_{16}N_2O_6$ 344.33

USP Nifedipine Nitrosophenylpyridine Analog RS

Dimethyl 4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.

$C_{17}H_{16}N_2O_5$ 328.33

Nifedipine Extended-Release Tablets**DEFINITION**

Nifedipine Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$). [NOTE—Nifedipine, when exposed to daylight and certain wavelengths of artificial light, readily converts to a nitrosophenylpyridine derivative. Exposure to UV light leads to the formation of a nitrophenylpyridine derivative. Perform assays and tests in the dark or under golden fluorescent or other low-actinic light. Use low-actinic glassware.]

IDENTIFICATION

- A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- B. ULTRAVIOLET ABSORPTION (197U)**
Standard stock solution and Sample stock solution: Prepare as directed in the *Assay*.

Standard solution: 0.02 mg/mL of USP Nifedipine RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Nominally 0.02 mg/mL of nifedipine in *Mobile phase* from the *Sample stock solution*

ASSAY

- PROCEDURE**

[NOTE—Conduct the *Assay* promptly after preparation of the *Standard solution* and the *Sample solution*.]

Mobile phase: Acetonitrile, methanol, and water (25:25:50)

Standard stock solution: 1 mg/mL of USP Nifedipine RS in methanol

Standard solution: 0.1 mg/mL of USP Nifedipine RS from the *Standard stock solution* in *Mobile phase*

Sample stock solution: Dissolve an amount equivalent to 420 mg of nifedipine from powdered Tablets in 130 mL of water in a 250-mL volumetric flask; or transfer the intact Tablets to a 400-mL, high-speed blender cup containing 130 mL of water. Homogenize until a uniform suspension is achieved (about 2 min), and transfer the suspension with the aid of a mixture of acetonitrile and methanol (1:1) to a 250-mL volumetric flask. Dilute with a mixture of acetonitrile and methanol (1:1) to volume, and stir for 30 min. Centrifuge the resulting suspension to obtain a clear supernatant.

Sample solution: Nominally 0.1 mg/mL of nifedipine prepared as follows. Transfer 3.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and filter. [NOTE—Reserve a portion of this solution for use as the *Sample solution* in the test for *Organic Impurities*.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Columns

Guard: 2.1-mm × 3-cm; packing L1

Analytical: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 25 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 4000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Nifedipine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of nifedipine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- DISSOLUTION (711)**

Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

Medium: Water; 50 mL

Apparatus 7: (See *Drug Release* (724).) 15–30 cycles/min. Do not use the reciprocating disk; use a 25-cm plexiglas rod, the perimeter of the Tablets being affixed to the rod with a water-insoluble glue. The solution containers are 25-mm test tubes, 150–200 mm in length, and the water bath is maintained at $37 \pm 0.5^\circ$. At the end of each specified test interval, the systems

are transferred to the next row of new test tubes containing 50 mL of fresh *Medium*.

Times: 4, 8, 12, 16, 20, and 24 h

Diluent: Methanol and water (1:1)

Standard solution: Transfer 50 mg of USP Nifedipine RS to a 100-mL volumetric flask. Dissolve in 50 mL of methanol, and dilute with water to volume. Quantitatively dilute this solution with *Diluent* to obtain solutions having suitable known concentrations.

Sample solution: Use portions of the solution under test, passed through a suitable filter of 0.4- μ m pore size, suitably diluted with methanol, and stepwise if necessary, with *Diluent* to obtain a final mixture consisting of equal parts of methanol and water.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 338 nm

Cell: 0.5 cm

Analysis: Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) released in the *Sample solution* at each 4-h interval from UV absorbances. [NOTE—For the 4-h time period, determine the absorbance at 456 nm, and use this determination to correct for excipient interference.]

Tolerances: See Table 1.

Table 1

Time (h)	Amount Dissolved ^a (%)
4	5–17
8	—
12	43–80
16	—
20	—
24	NLT 80

^a The amount dissolved is expressed in terms of the labeled Tablet strength rather than in terms of the labeled total contents.

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$), released at the times specified, conform to *Dissolution* (711), *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Solution A: Dissolve 330.9 g of dibasic sodium phosphate and 38 g of citric acid in water in a 1-L volumetric flask. Add 10 mL of phosphoric acid, and dilute with water to volume.

Medium: Mix 125.0 mL of *Solution A* and 1 L of 10% sodium lauryl sulfate solution, and dilute to 10 L. Adjust, if necessary, to a pH of 6.8; 900 mL.

Apparatus 2: 50 rpm, with sinkers. (See *Dissolution* (711), *Figure 2a*.)

Times: 3, 6, and 12 h

Mobile phase: Acetonitrile and water (7:3)

Standard stock solution: 1.11 mg/mL of USP

Nifedipine RS in methanol

Standard solution: 0.1 mg/mL of USP Nifedipine RS from the *Standard stock solution* in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 350 nm

Column: 4.0-mm \times 125-mm; 3- μ m packing L1

Temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved.

Tolerances: See Table 2.

Table 2

Time (h)	Amount Dissolved (%)
3	10–30
6	40–65
12	NLT 80

The percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$), released in vivo and dissolved at the times specified, conform to *Dissolution* (711), *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

For Tablets labeled to contain 30 mg of nifedipine:

Phase 1

Medium: 0.05 M phosphate buffer, pH 7.5; 900 mL

Apparatus 2: 100 rpm

Time: 1 h

Standard solution: 0.034 mg/mL of USP Nifedipine RS in *Medium*. [NOTE—If necessary, a volume of methanol, not exceeding 10% of the final volume, can be used to help solubilize nifedipine.]

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions(See *Ultraviolet-Visible Spectroscopy* (857).)**Mode:** UV**Analytical wavelength:** 238 nm**Cell:** 0.5 cm

Analysis: [NOTE—After the run, take the Tablet out of the dissolution vessel, adapt a sinker to it, and transfer the Tablet with the sinker to the dissolution vessel containing the *Medium* for Phase 2.] Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) released in Phase 1, using filtered portions of the *Sample solution*, in comparison with the *Standard solution*, using the *Medium* as the blank.

For Tablets labeled to contain 30 mg of nifedipine:
Phase 2

Medium: 0.5% Sodium lauryl sulfate in simulated gastric fluid without enzyme, pH 1.2; 900 mL

Apparatus 2: 100 rpm**Times:** 1, 4, 8, and 12 h

Standard solution: 0.034 mg/mL of USP Nifedipine RS in *Medium*. [NOTE—If necessary, a volume of methanol, not exceeding 10% of the final volume, can be used to help solubilize nifedipine.]

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions(See *Ultraviolet-Visible Spectroscopy* (857).)**Mode:** UV**Analytical wavelength:** 238 nm

Analysis: Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) released in Phase 2, using filtered portions of the *Sample solution*, in comparison with the *Standard solution*, using *Medium* as the blank.

Tolerances: See Table 3.**Table 3**

Time (h)	Amount Dissolved ^a (%)
1	NMT 30
4	30–55
8	NLT 60
12	NLT 80

^aFor each dosage unit, add the amount dissolved in phosphate buffer, pH 7.5 from Phase 1 to the amount dissolved at each time point in Phase 2.

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$), released in vivo and dissolved at the times specified, conform to *Dissolution* (711), *Acceptance Table 2*.

For Tablets labeled to contain 60 mg of nifedipine:
Phase 1

Medium: 0.05 M phosphate buffer, pH 7.5; 900 mL

Apparatus 2: 100 rpm**Time:** 25 min

Standard solution: 0.067 mg/mL of USP Nifedipine RS in *Medium*. [NOTE—If necessary, a volume of methanol, not exceeding 10% of the final volume, can be used to help solubilize nifedipine.]

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions(See *Ultraviolet-Visible Spectroscopy* (857).)**Mode:** UV**Analytical wavelength:** 238 nm

Analysis: [NOTE—After the run, take the Tablet out of the dissolution vessel, adapt a sinker to it, and transfer the Tablet with the sinker to the dissolution vessel containing the *Medium* for Phase 2.] Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) released in Phase 1, using filtered portions of the *Sample solution*, in comparison with the *Standard solution*, using the *Medium* as the blank.

For Tablets labeled to contain 60 mg of nifedipine:
Phase 2

Medium: 0.5% Sodium lauryl sulfate in simulated gastric fluid without enzyme, pH 1.2; 900 mL

Apparatus 2: 100 rpm**Times:** 1, 4, 8, and 12 h

Standard solution: 0.067 mg/mL of USP Nifedipine RS in *Medium*. [NOTE—If necessary, a volume of methanol, not exceeding 10% of the final volume, can be used to help solubilize nifedipine.]

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions(See *Ultraviolet-Visible Spectroscopy* (857).)**Mode:** UV**Analytical wavelength:** 238 nm

Analysis: Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) released in Phase 2, using filtered portions of the *Sample solution*, in comparison with the *Standard solution*, using the *Medium* as the blank.

Tolerances: See Table 4.**Table 4**

Time (h)	Amount Dissolved ^a (%)
1	NMT 30
4	40–70
8	NLT 70
12	NLT 80

^aFor each dosage unit, add the amount dissolved in phosphate buffer, pH 7.5 from Phase 1 to the amount dissolved at each time point in Phase 2.

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$), released in vivo and dissolved at the times specified, conform to *Dissolution* (711), *Acceptance Table 2*.

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution* Test 4.

Medium: 0.5% Sodium lauryl sulfate in simulated gastric fluid without enzyme, pH 1.2; 900 mL

Apparatus 2: 100 rpm**Times:** 1, 4, and 12 h

Standard solution: 0.067 mg/mL of USP Nifedipine RS for Tablets labeled to contain 60 mg, and 0.034 mg/mL of USP Nifedipine RS for Tablets labeled to contain 30 mg, in *Medium*. [NOTE—If necessary, a volume of methanol, not exceeding 10% of the final volume, can be used to help solubilize nifedipine.]

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions(See *Ultraviolet-Visible Spectroscopy* (857).)**Mode:** UV**Analytical wavelength:** UV 238 nm**Cell:** 1 cm

Analysis: Determine the amount of nifedipine ($C_{17}H_{18}N_2O_6$) released, using filtered portions of the *Sample solution*, in comparison with the *Standard solution*, using the *Medium* as the blank.

Tolerances: See Table 5 and Table 6.**Table 5**

For Tablets Labeled to Contain 30 mg of Nifedipine	
Time (h)	Amount Dissolved (%)
1	12–35
4	44–67
12	NLT 80

Table 6

For Tablets Labeled to Contain 60 mg of Nifedipine	
Time (h)	Amount Dissolved (%)
1	10–30
4	40–63
12	NLT 80

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$), released at the times specified, conform to *Dissolution* (711), *Acceptance Table 2*.

Test 5: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 5*.

Medium: Water; 50 mL

Apparatus 7: (See *Drug Release* (724).) Use a 25-cm plexiglas rod, the perimeter of the Tablets being affixed to the rod with a water-insoluble glue; 30 dips/min. The solution containers are 25-mm test tubes, 150–200 mm in length, and the water bath is maintained at $37 \pm 0.5^\circ$.

Times: 4, 12, and 24 h

Diluent A: Methanol and acetonitrile (1:1)

Diluent B: Diluent A and water (1:1)

Standard stock solution: 50 mg of USP Nifedipine RS in Diluent A and water (50:50)

Standard solutions: 0.01, 0.05, and 0.20 mg/mL solutions, from the *Standard stock solution* in Diluent B, that are used at 4-, 12-, and 24-h sampling

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 238 nm

Cell: 0.5 cm

Analysis: [NOTE—For the 4-h time period, filter the solution under test, and determine the absorbance at 456 nm. Use this absorbance value to correct for excipient interference at the other time points.] Determine the amount of nifedipine released at each interval on portions of the *Sample solution* passed through a suitable filter of 0.45- μ m pore size, suitably diluted, if necessary, with Diluent A and water to obtain a final mixture of water, methanol, and acetonitrile (2:1:1), in comparison with the appropriate *Standard solution*, using Diluent B as the blank.

Tolerances: See Table 7.

Table 7

Time (h)	Amount Dissolved (%)
4	NMT 14
12	39–75
24	NLT 75

The cumulative percentages of the labeled amount of nifedipine, released in vivo and dissolved at the times specified, conform to *Dissolution* (711), *Acceptance Table 2*.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium: Simulated gastric fluid without enzyme containing 0.5% of sodium lauryl sulfate, pH 1.2; 900 mL, deaerated

Apparatus 1: 100 rpm

Times: 1, 4, and 12 h

Standard stock solution: 0.33 mg/mL of USP Nifedipine RS in methanol

Standard solution: Quantitatively dilute the *Standard stock solution* with *Medium* to obtain a solution having a concentration of about 0.033 mg/mL.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 329 nm

Cell: 0.5 cm

Blank: *Medium*

Tolerances: See Table 8.

Table 8

Time (h)	Amount Dissolved (%)
1	NMT 15
4	20%–40
12	NLT 80

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 7: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

Medium: Simulated gastric fluid without enzyme containing 0.5% sodium lauryl sulfate, pH 1.2; 900 mL

Apparatus 2: 100 rpm, with three-prong sinker

Times: 1, 4, and 12 h

Standard solution: (L/900) mg/mL of USP Nifedipine RS in *Medium*, where L is the label claim, in mg/Tablet, of nifedipine. A small amount of methanol, not exceeding 6%–7% of the final volume of the first dilution, can be used to solubilize nifedipine.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 238 nm

Cell: 1 mm, flow cell

Blank: *Medium*

Tolerances: See Table 9.

Table 9

Time (h)	Amount Dissolved (%)
1	NMT 15
4	25–50
12	NLT 80

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 8: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

Acid stage medium: Simulated gastric fluid without enzyme containing 3% polysorbate 80, pH 1.2; 250 mL

Apparatus 3: 20 dpm, 20-mesh polypropylene screen on the bottom; 1 min drip time. The Tablet is automatically transferred by the apparatus to the next set of vessels for each time point.

Time: 1 h

Buffer stage medium: 0.01 M sodium phosphate buffer, pH 6.8, containing 3% polysorbate 80 (dissolve 8.3 g of monobasic sodium phosphate and 1 g of sodium hydroxide in 6 L of water, adjust with either diluted sodium hydroxide or phosphoric acid to a pH of 6.8 ± 0.05 , and add 180 g of polysorbate 80); 250 mL

Times: 2, 8, 12, and 24 h

Mobile phase: Acetonitrile, methanol, and water (35:35:30)

Standard stock solution: 1 mg/mL of USP Nifedipine RS in *Buffer stage medium*. An amount of methanol,

about 40% of the final volume, can be used to dissolve nifedipine.

Standard solution: (L/1000) mg/mL in *Buffer stage medium*, from the *Standard stock solution*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 338 nm

Column: 4.6-mm × 25-cm; packing L1

Temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 4000 theoretical plates

Tailing factor: NMT 1.7

Relative standard deviation: NMT 2.0%

Analysis: Calculate the percentage of the labeled amount of nifedipine dissolved at each time point.

At 1 h:

$$D_1 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

At 2 h:

$$D = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$D_2 = D_1 + D$$

At 8 h:

$$D = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$D_8 = D_2 + D$$

At 12 h:

$$D = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$D_{12} = D_8 + D$$

At 24 h:

$$D = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$D_{24} = D_{12} + D$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 250 mL

Tolerances

Acid stage: NMT 5% of the labeled amount of nifedipine is dissolved in 1 h.

Buffer stage: See Table 10.

Table 10

Time (h)	Amount Dissolved (%)
1	NMT 5
2	0–10
8	25–60
12	45–85
24	NLT 80

The cumulative percentages of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 9: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.

Medium: 0.03 M phosphate/citrate buffer, pH 6.8 with 1% sodium lauryl sulfate. (To a solution of 4.1 g/L of dibasic sodium phosphate and 0.475 g/L of citric acid monohydrate in water, add 10 g/L of sodium lauryl sulfate. Adjust if necessary, with phosphoric acid to a pH of 6.8.); 900 mL

Apparatus 2: 50 rpm, with a suitable sinker

Times: 3, 6, and 12 h

Standard stock solution: 0.33 mg/mL of USP Nifedipine RS in methanol

Standard solution: Prepare the corresponding USP Nifedipine RS solutions in *Medium* as directed in Table 11.

Table 11

Tablet Strength (mg)	Concentration (mg/mL)
30	0.033
60	0.066
90	0.099

Sample solution: Pass a portion of the solution under test at each time point through a suitable filter.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 346 nm

Cell: 1 cm

Blank: *Medium*

Analysis

Samples: *Standard solutions* and *Sample solution*

Calculate the concentration (C_i) of nifedipine ($C_{17}H_{18}N_2O_6$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S$$

A_U = absorbance of the *Sample solution* at each time point

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Nifedipine RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_3)] + (C_1 \times V_3)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_3)]] + [(C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

C_i = concentration of nifedipine in the *Sample solution* at the specified time point (i) (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim (mg/Tablet)

V_3 = volume of the *Sample solution* withdrawn at each time point (i) (mL)

Tolerances: See Table 12.

Table 12

Time Point (i)	Time (h)	Amount Dissolved (%)	
		Tablets labeled to contain 30 mg and 60 mg of Nifedipine	Tablets labeled to contain 90 mg of Nifedipine
1	3	15–40	10–35
2	6	43–73	40–65
3	12	NLT 80	NLT 80

The percentages of the labeled amount of Nifedipine ($C_{17}H_{16}N_2O_6$) dissolved at the times specified conform to Dissolution (711), Acceptance Table 2.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

[NOTE—Conduct this test promptly after preparation of the *Standard nifedipine solution* and the *Sample solution*.]

Mobile phase: Acetonitrile, methanol, and water (25:25:50)

Quantitative limit stock solution A: 1 mg/mL of USP Nifedipine Nitrophenylpyridine Analog RS in methanol

Quantitative limit solution A: 6 µg/mL of USP Nifedipine Nitrophenylpyridine Analog RS from *Quantitative limit stock solution A* in *Mobile phase*

Quantitative limit stock solution B: 1 mg/mL of USP Nifedipine Nitrosophenylpyridine Analog RS in methanol

Quantitative limit solution B: 1.5 µg/mL of USP Nifedipine Nitrosophenylpyridine Analog RS from *Quantitative limit stock solution B* in *Mobile phase*

Standard nifedipine stock solution: 1 mg/mL of USP Nifedipine RS in methanol

Standard nifedipine solution: 0.3 mg/mL of USP Nifedipine RS from *Standard nifedipine stock solution* in *Mobile phase*

System suitability solution: *Quantitative limit solution A*, *Quantitative limit solution B*, and *Standard nifedipine solution* (1:1:1)

Standard solution: *Mobile phase*, *Quantitative limit solution A*, and *Quantitative limit solution B* (1:1:1)

[NOTE—Each mL of this solution contains about 2 µg of USP Nifedipine Nitrophenylpyridine Analog RS and 0.5 µg of USP Nifedipine Nitrosophenylpyridine Analog RS.]

Sample solution: Use a portion of the *Sample solution* prepared as directed in the Assay.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Columns

Guard: 2.1-mm × 3-cm; packing L1

Analytical: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between the nitrophenylpyridine analog and nitrosophenylpyridine analog peaks; NLT 1.0 between the nitrosophenylpyridine analog and nifedipine peaks

Relative standard deviation: NMT 10% for each analog

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each analog in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each analog from the *Sample solution*

r_S = peak response of each analog from the *Standard solution*

C_S = concentration of the appropriate analog USP Reference Standard in the *Standard solution* (µg/mL)

C_U = nominal concentration of nifedipine in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 2.0% of nifedipine nitrophenylpyridine analog and NMT 0.5% of nifedipine nitrosophenylpyridine analog, both relative to the nifedipine content

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

- **LABELING:** The labeling indicates the *Dissolution Test* with which the product complies.

- **USP REFERENCE STANDARDS (11)**

USP Nifedipine RS

USP Nifedipine Nitrophenylpyridine Analog RS

Dimethyl 4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.

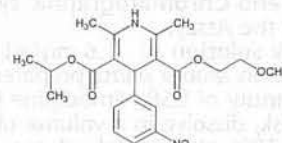
$C_{17}H_{16}N_2O_6$ 344.33

USP Nifedipine Nitrosophenylpyridine Analog RS

Dimethyl 4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate.

$C_{17}H_{16}N_2O_5$ 328.33

Nimodipine



$C_{21}H_{26}N_2O_7$ 418.44

3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-, 2-methoxyethyl 1-methylethyl ester; Isopropyl 2-methoxyethyl 1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate [66085-59-4].

DEFINITION

Nimodipine contains NLT 98.0% and NMT 102.0% of nimodipine ($C_{21}H_{26}N_2O_7$), calculated on the dried basis.

[NOTE—Throughout the following procedures, protect samples, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

- **B.** The retention time of the *Sample solution* corresponds to that of *Standard solution A*, as obtained in the test for *Organic Impurities*.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, tetrahydrofuran, and water (200:200:600)

Standard solution: 0.3 mg/mL of USP Nimodipine RS in *Mobile phase* prepared as follows. Transfer a quantity

of USP Nimodipine RS to a suitable volumetric flask, dissolve in a volume of tetrahydrofuran equivalent to 10% of the total volume, and dilute with *Mobile phase* to volume.

Sample solution: 0.3 mg/mL of Nimodipine in *Mobile phase* prepared as follows. Transfer a quantity of Nimodipine to a suitable volumetric flask, dissolve in a volume of tetrahydrofuran equivalent to 10% of the total volume, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 12.5-cm; 5-μm packing L1

Temperature: 40°

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nimodipine ($C_{21}H_{26}N_2O_7$) in the portion of Nimodipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nimodipine from the *Sample solution*

r_S = peak response of nimodipine from the *Standard solution*

C_S = concentration of USP Nimodipine RS in the *Standard solution* (mg/mL)

C_U = concentration of Nimodipine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• ORGANIC IMPURITIES

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*.

Standard stock solution A: 1.6 mg/mL of USP Nimodipine RS in *Mobile phase* prepared as follows. Transfer a quantity of USP Nimodipine RS to a suitable volumetric flask, dissolve in a volume of tetrahydrofuran equivalent to 10% of the total volume, and dilute with *Mobile phase* to volume.

Standard stock solution B: 0.8 mg/mL each of USP Nimodipine RS and USP Nimodipine Related Compound A RS in *Mobile phase* prepared as follows. Transfer quantities of USP Nimodipine RS and USP Nimodipine Related Compound A RS to a suitable volumetric flask, dissolve in a volume of tetrahydrofuran equivalent to 10% of the total volume, and dilute with *Mobile phase* to volume.

Standard solution A: 3.2 μg/mL of USP Nimodipine RS from *Standard stock solution A* in *Mobile phase*

Standard solution B: 1.6 μg/mL each of USP Nimodipine RS and USP Nimodipine Related Compound A RS from *Standard stock solution B* in *Mobile phase*

Sample solution: 1.6 mg/mL of Nimodipine prepared as follows. Dissolve 40 mg of Nimodipine in 2.5 mL of tetrahydrofuran, and dilute with *Mobile phase* to 25 mL.

System suitability

Sample: *Standard solution B*

[NOTE—The relative retention times for nimodipine related compound A and nimodipine are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between nimodipine related compound A and nimodipine

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of nimodipine related compound A in the portion of Nimodipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nimodipine related compound A from the *Sample solution*

r_S = peak response of nimodipine related compound A from *Standard solution B*

C_S = concentration of USP Nimodipine Related Compound A RS in *Standard solution B* (μg/mL)

C_U = concentration of Nimodipine in the *Sample solution* (μg/mL)

Acceptance criteria: NMT 0.1% of nimodipine related compound A

Calculate the percentage of any other impurity in the portion of Nimodipine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of nimodipine from *Standard solution A*

C_S = concentration of USP Nimodipine RS in *Standard solution A* (μg/mL)

C_U = concentration of Nimodipine in the *Sample solution* (μg/mL)

Acceptance criteria

Individual impurities: NMT 0.2% of any other impurity

Total impurities: NMT 0.5%

SPECIFIC TESTS

• **OPTICAL ROTATION**, *Specific Rotation* (781S)

Sample solution: 50 mg/mL in acetone

Acceptance criteria: −10° to +10°

• **LOSS ON DRYING** (731)

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

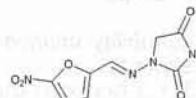
USP Nimodipine RS

USP Nimodipine Related Compound A RS

2-Methoxyethyl-1-methylethyl-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate.

$C_{21}H_{24}N_2O_7$ 416.42

Nitrofurantoin



$C_8H_6N_4O_5$ (anhydrous) 238.16

$C_8H_6N_4O_5 \cdot H_2O$ 256.18

2,4-Imidazolidinedione, 1-[[[(5-nitro-2-furyl)methylene]-amino]-;

1-[(5-nitrofurfurylidene)amino]hydantoin [67-20-9].

Monohydrate [17140-81-7].

DEFINITION

Nitrofurantoin is anhydrous or contains one molecule of water of hydration. It contains NLT 98.0% and NMT 102.0% of $C_8H_6N_4O_5$, calculated on the anhydrous basis. [NOTE—Nitrofurantoin and solutions of it are discolored by alkali and by exposure to light and are decomposed upon contact with metals other than stainless steel and aluminum.]

IDENTIFICATION• **A. INFRARED ABSORPTION (197M)**

Sample: Previously dried at 140° for 30 min

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 500 mL of water. Add about 30 mL of 1.0 N sodium hydroxide sufficient to adjust to a pH of 7.0, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (12:88)

Internal standard solution: 1 mg/mL of acetanilide in water

Standard solution: 0.56 mg/mL of USP Nitrofurantoin RS prepared as follows. Dissolve 50 mg of USP Nitrofurantoin RS in 40.0 mL of dimethylformamide, and add 50.0 mL of *Internal standard solution*.

Sample solution: 0.56 mg/mL of Nitrofurantoin prepared as directed for *Standard solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

[NOTE—Adjust the operating parameters so that the retention time of the nitrofurantoin peak is about 8 min and the peak heights are about half full-scale.]

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Injection size: 5–10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between the acetanilide and nitrofurantoin peaks

Relative standard deviation: NMT 2.0% determined from the ratio of the peak responses

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nitrofurantoin ($C_8H_6N_4O_5$) in the portion of Nitrofurantoin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = internal standard ratio (peak response of nitrofurantoin/peak response of acetanilide) from the *Sample solution*

R_S = internal standard ratio (peak response of nitrofurantoin/peak response of acetanilide) from the *Standard solution*

C_S = concentration of USP Nitrofurantoin RS in the *Standard solution* (mg/mL)

C_U = concentration of Nitrofurantoin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES• **LIMIT OF NITROFURFURAL DIACETATE**

Standard solution: 100 µg/mL of USP Nitrofurfural Diacetate RS in dimethylformamide and acetone (1:10)

Sample solution: Dissolve 100 mg of Nitrofurantoin in 1 mL of dimethylformamide, and dilute with acetone to 10.0 mL.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Chloroform and methanol (9:1)

Spray reagent: Dissolve 0.75 g of phenylhydrazine hydrochloride in 50 mL of water, and decolorize with activated charcoal. Add 25 mL of hydrochloric acid, and mix with water to produce 200 mL.

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter. Develop in the *Developing solvent system* until the solvent front has moved three-fourths the length of the plate, allow to air-dry for 5 min, and heat the plate at 105° for 5 min. While it is still warm, locate the spots by spraying the plate with *Spray reagent*.

Acceptance criteria: Any spot produced by the *Sample solution*, at an R_f value of 0.7, is not greater in size or intensity than that produced by the *Standard solution* at the same R_f value: NMT 1.0% of nitrofurfural diacetate is found.

• **LIMIT OF NITROFURAZONE**

Buffer: Prepare as directed in the *Assay*.

Mobile phase: Tetrahydrofuran and *Buffer* (10:90)

System suitability stock solution: 5.0 µg/mL each of USP Nitrofurazone RS and USP Nitrofurantoin RS in dimethylformamide

System suitability solution: 0.5 µg/mL each of USP Nitrofurazone RS and USP Nitrofurantoin RS in *Mobile phase* from *System suitability stock solution*

Standard stock solution: 5.0 µg/mL of USP Nitrofurazone RS in dimethylformamide

Standard solution: 0.5 µg/mL of USP Nitrofurazone RS in water from *Standard stock solution*

Sample solution: Dissolve 100 mg of Nitrofurantoin in 2.0 mL of dimethylformamide, and add 20.0 mL of water. Mix, and allow to stand for 15 min to allow a precipitate to form. Pass a portion of the solution through a suitable nylon filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

[NOTE—Adjust the operating parameters so that the retention time of the nitrofurazone peak is about 10.5 min and its height is about 0.1 half full-scale.]

Mode: LC

Detector: UV 375 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.6 mL/min

Injection size: 60–100 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between the nitrofurazone and nitrofurantoin peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% determined from the peak height, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The height of any peak appearing in the *Sample solution* at a retention time corresponding to that of the main peak from the *Standard solution* is NMT the height of the main peak from the *Standard solution* (NMT 0.01%).

SPECIFIC TESTS• **WATER DETERMINATION**, Method III (921)

Analysis: Dry a sample at 140° for 30 min.

Acceptance criteria: For the anhydrous form, it loses NMT 1.0% of its weight; for the hydrous form, it loses 6.5%–7.5% of its weight.

• **SPECIFIC SURFACE AREA** (846) (where it is labeled as being in the form of macrocrystals)

Sample: Outgas a portion of the powder to be placed under test at 150° for 10 min at ambient pressure with nitrogen.

Acceptance criteria: 0.045–0.20 m²/g

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.• **LABELING:** Label it to indicate whether it is anhydrous or hydrous. Nitrofurantoin in the form of macrocrystals is so labeled. The labeling states the specific surface area and which method, specified under *Specific Surface Area* (846), is used.• **USP REFERENCE STANDARDS** (11)

USP Nitrofurantoin RS

USP Nitrofurazone RS

USP Nitrofurfural Diacetate RS

C₉H₉NO₇ 243.17

Nitrofurantoin Capsules**DEFINITION**

Nitrofurantoin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of nitrofurantoin (C₈H₆N₄O₅).

IDENTIFICATION• **A. INFRARED ABSORPTION**

Sample: Add 10 mL of 6 N acetic acid to a quantity of the contents of Capsules equivalent to 100 mg of nitrofurantoin. Boil the solution for a few min, and filter while hot. Cool to room temperature, collect the precipitate of nitrofurantoin, and dry at 105° for 1 h.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the precipitate so obtained exhibits maxima only at the same wavelength as that of a similar solution of USP Nitrofurantoin RS.

• **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**ASSAY**• **PROCEDURE**

Solution A: Dissolve 6.8 g of monobasic potassium phosphate in 500 mL of water. Add a volume of 1.0 N sodium hydroxide (about 30 mL) sufficient to adjust to a pH of 7.0, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Solution A* (3:22)

Internal standard solution: 1 mg/mL of acetanilide in water

Standard solution: Dissolve 50 mg of USP Nitrofurantoin RS in 40.0 mL of dimethylformamide, and add 50.0 mL of *Internal standard solution*.

Sample solution: Transfer, as completely as possible, the contents of 20 Capsules to a 500-mL flask. Place the emptied Capsules in a beaker, add 25 mL of dimethylformamide, and agitate for 1 min. Decant into the flask containing the Capsule contents. Rinse the emptied Capsules with another two 25-mL portions of dimethylformamide, and decant into the flask. Add sufficient dimethylformamide to bring the volume to about 250 mL. Insert the stopper in the flask, and shake by mechanical means for 15 min. Dilute with dimethylformamide to volume, and mix. If necessary, the sample may be homogenized using a disperser. Pass

through a medium-porosity, sintered-glass filter into a suitable flask. Transfer an aliquot, equivalent to 50 mg of nitrofurantoin, to a flask. Add an accurately measured volume of dimethylformamide to bring the volume in the flask to 40.0 mL. To the flask add 50.0 mL of *Internal standard solution*, mix, and cool to room temperature. Pass a portion of the solution through a nylon filter of 0.45-μm pore size, discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Injection volume: 5–10 μL

System suitability

Sample: *Standard solution*

[NOTE—Adjust the operating parameters so that the retention time of the nitrofurantoin peak is about 8 min, and the peak heights are about half full-scale.]

Suitability requirements

Resolution: NLT 3.0 between acetanilide and nitrofurantoin

Relative standard deviation: NMT 2.0%, determined from peak response ratios of replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nitrofurantoin (C₈H₆N₄O₅) in the portion of the powder included in the sample aliquot:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio from the *Sample solution*

R_S = peak response ratio from the *Standard solution*

C_S = concentration of USP Nitrofurantoin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)

Test 1 (where it is labeled as containing nitrofurantoin macrocrystals)

Medium: pH 7.2 (± 0.05) phosphate buffer; 900 mL

Apparatus 1: 100 rpm

Times: 1, 3, and 8 h

Standard solution: USP Nitrofurantoin RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

Blank: *Medium*

Instrumental conditions

Mode: UV

Analytical wavelength: 375 nm

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved
1	20%–60%
3	NLT 45%
8	NLT 60%

The percentage of the labeled amount of nitrofurantoin (C₈H₆N₄O₅) dissolved at the 1-h point conforms to *Acceptance Table 2* in *Dissolution* (711), and the percentages dissolved at the 3- and 8-h points conform to the criteria for the final test time in *Acceptance Table 2* in (711).

Test 2 (where it is labeled as containing both nitrofurantoin macrocrystalline and monohydrate forms): If the

product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Acid medium: 0.01 N hydrochloric acid for 1 h; 900 mL

pH 7.5 buffer medium: Prepare a pH 7.5 buffer concentrate by dissolving 62.2 g of potassium hydroxide and 129.3 g of monobasic potassium phosphate in water, dilute with water to 1 L, and mix. After 1 h, change the *Acid medium* to *pH 7.5 buffer medium* by adding 50 mL of pH 7.5 buffer concentrate, and run for an additional 6 h.

Apparatus 2: 100 rpm, with sinkers made of Teflon-coated steel wire prepared by forming a coil approximately 22 mm long from a 13-cm length of 20-gauge wire (see Figure 1)

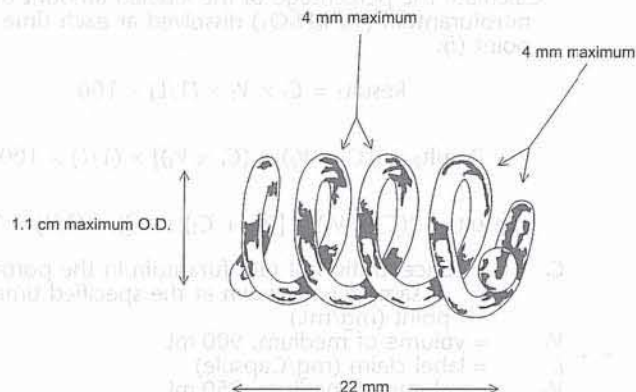


Figure 1. Sinkers.

Times: 1, 3, and 7 h

Acid-stage standard solution: 0.025 mg/mL of USP Nitrofurantoin RS in *Acid medium*

Buffer-stage standard solution: 0.075 mg/mL of USP Nitrofurantoin RS in *pH 7.5 buffer medium*

Instrumental conditions

Mode: UV

Analytical wavelength: 375 nm

Analysis: Calculate the percentages of the labeled amount (Q) of nitrofurantoin ($C_8H_6N_4O_5$) dissolved from UV absorbances at the isosbestic wavelength at about 375 nm on filtered portions of each solution under test, suitably diluted, if necessary, with *Acid medium* or *pH 7.5 buffer medium* when appropriate, in comparison with the appropriate *Standard solution*.

Tolerances: See Table 2.

Table 2

Time (h)	Amount Dissolved (Individual)	Amount Dissolved (Mean)
1	2%–16%	5%–13%
3	27%–69%	39%–56%
7	NLT 68%	NLT 81%

The percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at the specified times conform to Table 3.

Table 3

Level	Number Tested	Criteria
L ₁	12	The mean percentage of dissolved label claim lies within the range for the means at each interval and is NLT the stated amount at the final test time. All individual values lie within the ranges for the individuals at each interval and are NLT the stated amount at the final test time.
L ₂	12	The mean percentage of dissolved label claim lies within the range for the means at each interval and is NLT the stated amount at the final test time. NMT 2 of the 24 individual values lie outside the stated ranges for individuals at each interval, and NMT 2 of 24 are less than the stated amount at the final test time.

Test 3 (where it is labeled as containing both nitrofurantoin macrocrystalline and monohydrate forms): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Acid medium, pH 7.5 buffer medium, Apparatus 2, Times, Acid-stage standard solution, Buffer-stage standard solution, and Analysis: Proceed as directed in Test 2.

Tolerances: See Table 4.

Table 4

Time (h)	Amount Dissolved (Individual)	Amount Dissolved (Mean)
1	2%–16%	5%–13%
3	50%–80%	55%–75%
7	NLT 85%	NLT 90%

The percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at the specified times conform to Acceptance Table 2 in (711).

Test 4 (where it is labeled as containing both nitrofurantoin macrocrystalline and monohydrate forms): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Acid medium: 0.01 N hydrochloric acid for 1 h; 900 mL, deaerated

pH 7.5 buffer medium: Prepare a pH 7.5 buffer concentrate by dissolving 62.2 g of potassium hydroxide and 129.3 g of monobasic potassium phosphate in water, dilute with water to 1 L, and mix. After 1 h change the *Acid medium* to *pH 7.5 buffer medium* by adding 50 mL of pH 7.5 buffer concentrate, and run for an additional 9 h.

Apparatus 2: 100 rpm, with helix sinkers

Times: 1, 3, and 10 h

Standard stock solution: Transfer 25 mg of USP Nitrofurantoin RS to a 10-mL volumetric flask. Add 7.5 mL of dimethylformamide, and sonicate until dissolved. Allow to cool to room temperature, and dilute with dimethylformamide to volume.

Acid-stage standard solution: Dilute 2.0 mL of the *Standard stock solution* with *Acid medium* to 200 mL.

Buffer-stage standard solution: Transfer 3.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with *pH 7.5 buffer medium* to volume.

Stock capsule shell blank: Place 10 empty, clean Capsules into a 900-mL volumetric flask, and add 800 mL of *Acid medium*. Gently heat to $37 \pm 0.5^\circ$, and stir until all the Capsules are dissolved. Allow to cool to room temperature, and dilute with *Acid medium* to volume.

Buffer-stage capsule shell blank: Transfer 100.0 mL of the *Stock capsule shell blank* to a 1000-mL volumetric flask. Add 56 mL of pH 7.5 buffer medium, dilute with *Acid medium* to volume, and mix. Filter, using the same filter as for the *Sample solution*.

Sample solution: Pass portions of the solution under test through a 1.2- μ m glass/0.45- μ m polyethersulfone combination filter, discarding the first few mL.

Instrumental conditions

Mode: UV

Analytical wavelength: 375 nm

Analysis: Calculate the percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved from portions of the *Sample solution* in comparison with the appropriate *Acid-stage standard solution* or *Buffer-stage standard solution*. Correct for the appropriate capsule shell blank absorbance, using a 0.1-cm cell, and the appropriate medium as the blank.

Tolerances: See Table 5.

Table 5

Time (h)	Amount Dissolved
1	NMT 25%
3	25%–50%
10	NLT 80%

The percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at the specified times conform to Acceptance Table 2 in (711).

Test 5 (where it is labeled as containing both nitrofurantoin macrocrystalline and monohydrate forms): If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 5.

Acid medium: 0.01 N hydrochloric acid for 1 h; 900 mL, deaerated

Buffer concentrate: 60 g/L of potassium hydroxide and 129.3 g/L of monobasic potassium phosphate in water

pH 7.5 buffer medium: Prepare by adding 60 mL of *Buffer concentrate* to 890 mL of *Acid medium*.

Apparatus 2: 100 rpm, with Teflon-coated sinkers and paddles

Times: 1, 3, and 7 h

Standard stock solution: 2.48 mg/mL of USP Nitrofurantoin RS in acetonitrile. Sonicate using 50% of the final volume of acetonitrile to dissolve. Use an amber volumetric flask.

Acid-stage standard solution: 24.8 μ g/mL of USP Nitrofurantoin RS in *Acid medium* from *Standard stock solution*. Use an amber volumetric flask.

Buffer-stage standard solution: 74.4 μ g/mL of USP Nitrofurantoin RS in pH 7.5 buffer medium from *Standard stock solution*. Use an amber volumetric flask.

Acid-stage sample solution: After 1 h, collect 10 mL of the solution under test, and pass through a 0.45- μ m PVDF filter, discarding the first 5 mL of the filtrate.

Buffer-stage sample solution: After removing 10 mL of *Acid medium*, add 60 mL of pH 7.5 buffer medium. The pH of the resulting medium should be about 7.5. Continue the dissolution for another 2 h and 6 h. Collect 10 mL at each time point, and pass through a 0.45- μ m PVDF filter, discarding the first 5 mL of the filtrate.

Acid-stage blank: Use *Acid medium*.

Buffer-stage blank: Use pH 7.5 buffer medium.

Instrumental conditions

Mode: UV

Analytical wavelength: 375 nm

Cell: 0.5 cm for acid-stage and 0.1 cm for buffer-stage

Analysis

Samples: *Acid-stage standard solution*, *Buffer-stage standard solution*, *Acid-stage sample solution*, *Buffer-stage sample solution*, *Acid-stage blank*, and *Buffer-stage blank*

Calculate the concentration (C_i) of nitrofurantoin ($C_8H_6N_4O_5$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_u/A_s) \times C_s$$

A_u = absorbance of the *Sample solution*

A_s = absorbance of the *Standard solution*

C_s = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V_i \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V_2) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V_3) + [(C_2 + C_1) \times V_5]\} \times (1/L) \times 100$$

C_i = concentration of nitrofurantoin in the portion of sample withdrawn at the specified time point (mg/mL)

V_i = volume of medium, 900 mL

L = label claim (mg/Capsule)

V_2 = volume of medium, 950 mL

V_3 = volume of the *Sample solution* withdrawn at each time point, 10 mL

V_5 = volume of medium, 940 mL

Tolerances: See Table 6.

Table 6

Time Point (i)	Time (h)	Amount Dissolved (Individual)	Amount Dissolved (Mean)
1	1	NMT 12%	NMT 12%
2	3	NLT 80%	80%–100%
3	7	NLT 85%	NLT 90%

The percentages of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) dissolved at the specified times conform to Table 7.

Table 7

Level	Number Tested	Criteria
		The mean percentage of dissolved label claim lies within the range for the means at each interval and is NLT the stated amount at the final test time. All individual values lie within the ranges for the individuals at each interval and are NLT the stated amount at the final test time.
L_1	12	

Table 7 (Continued)

Level	Number Tested	Criteria
L ₂	12	If the requirements of level L ₁ are not met, test another twelve (12) Capsules. The requirements are met if the mean percentage of dissolved label claim of all 24 Capsules tested lies within the range for the means at each interval and is NLT the stated amount at the final test time. NMT 2 of the 24 individual values of Capsules lie outside the stated range for individuals at each interval, and NMT 2 of 24 Capsules are less than the stated amount at the final test time.

• UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Solution A, Mobile phase, Internal standard solution, Standard solution, Chromatographic system, and Analysis: Proceed as directed in the Assay.

Sample solution: Transfer the contents of 1 Capsule to a suitable flask, and add a volume of dimethylformamide to obtain a solution having a concentration of about 1.2 mg/mL of nitrofurantoin. Shake the flask for 15 min. If necessary, the sample may be homogenized, using a disperser. In the case of a 50- or 100-mg Capsule, transfer 40.0 mL of this solution to a suitable flask, add 50.0 mL of *Internal standard solution*, mix, and cool to room temperature. Pass a portion of the solution through a nylon filter of 0.45- μ m pore size, discarding the first few mL of the filtrate. In the case of a 25-mg Capsule, transfer 20.0 mL of the solution to a suitable flask, and add 25.0 mL of *Internal standard solution* instead of 50.0 mL.

Acceptance criteria: Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES: LIMIT OF NITROFUZAZONE

Solution A: Prepare as directed in the Assay.

Mobile phase: Tetrahydrofuran and *Solution A* (1:9)

System suitability stock solution: 5.0 μ g/mL each of nitrofurazone and nitrofurantoin in dimethylformamide

System suitability solution: *System suitability stock solution* and *Mobile phase* (1:10)

Standard stock solution: 5.0 μ g/mL of USP Nitrofurazone RS in dimethylformamide

Standard solution: Transfer 2.0 mL of the *Standard stock solution* into a glass-stoppered flask, add 20.0 mL of water, and mix.

Sample solution: Transfer a portion of Capsule contents equivalent to 100 mg of nitrofurantoin into a 25-mL glass-stoppered flask. Add 2.0 mL of dimethylformamide, and shake for 5 min. Add 20.0 mL of water, mix, and allow to stand for 15 min. Pass a portion of the mixture through a nylon filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 375 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1.6 mL/min

Injection volume: 60–100 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—Adjust the operating parameters so that the nitrofurazone peak in the chromatogram of the *Standard solution* has a retention time of about 10.5 min and a height of about 0.1 full-scale.]

Suitability requirements

Resolution: NLT 4.0 between the nitrofurazone and nitrofurantoin peaks, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The height of any peak from the *Sample solution* at a retention time corresponding to that of the main peak from the *Standard solution* is NMT the height of the main peak from the *Standard solution*. NMT 0.01% of nitrofurazone is found.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Capsules that contain the macrocrystalline form of nitrofurantoin are so labeled. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
 - USP Nitrofurantoin RS
 - USP Nitrofurazone RS

Nitrofurantoin Oral Suspension

DEFINITION

Nitrofurantoin Oral Suspension is a suspension of Nitrofurantoin in a suitable aqueous vehicle. It contains, in each 100 mL, NLT 460 mg and NMT 540 mg of nitrofurantoin ($C_8H_6N_4O_5$).

IDENTIFICATION

• A. INFRARED ABSORPTION

Sample solution: 10 mL of Oral Suspension in 15 mL of acetone

Analysis: Warm the *Sample solution* to 50°, with stirring, to coagulate the excipients. Filter, evaporate the acetone with the aid of a warm air blast nearly to dryness, add 10 mL of acetic acid, heat to boiling, and filter while hot. Cool the filtrate to room temperature. Filter the precipitated nitrofurantoin, and dry at 105° for 1 h.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the precipitate obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Nitrofurantoin RS.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 500 mL of water. Add about 30 mL of 1.0 N sodium hydroxide sufficient to adjust to a pH of 7.0, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (12:88)

Internal standard solution: 0.065 mg/mL of acetanilide in *Mobile phase*

Standard stock solution: 0.25 mg/mL of USP Nitrofurantoin RS prepared as follows. Transfer the required amount in suitable volumetric flask, and dissolve in 50% of the final volume of dimethylformamide and 20% of the final volume of water. Cool to room temperature, and dilute with dimethylformamide to volume.

Standard solution: Transfer 4.0 mL of *Standard stock solution* to a glass-stoppered flask, and add 15.0 mL of *Internal standard solution*.

Sample stock solution: Nominally 0.25 mg/mL of nitrofurantoin prepared as follows. Transfer a volume of

Oral Suspension to a suitable volumetric flask, add 20% of the final volume of water, and mix. Add 50% of the final volume of dimethylformamide, and shake the flask for 20 min. Cool to room temperature, and dilute with dimethylformamide to volume. Centrifuge a portion of the solution, and use the supernatant to prepare the *Sample solution*.

Sample solution: Transfer 4.0 mL of *Sample stock solution* to a glass-stoppered flask, add 15.0 mL of *Internal standard solution*, and mix. Pass a portion of the solution through a 5- μ m pore size polytetrafluoroethylene filter, discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

[NOTE—Adjust the operating parameters so that the retention time of the nitrofurantoin peak is about 8 min and its peak height is about half-full scale.]

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection size: 15 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.5 between the acetanilide and nitrofurantoin peaks

Relative standard deviation: NMT 2.0% determined from the ratio of the peak responses

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity per volume, in mg/100 mL, of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$) in the Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times C_S \times D$$

R_U = internal standard ratio (peak response of nitrofurantoin/peak response of acetanilide) from the *Sample solution*

R_S = internal standard ratio (peak response of nitrofurantoin/peak response of acetanilide) from the *Standard solution*

C_S = concentration of USP Nitrofurantoin RS in the *Standard solution* (mg/mL)

D = dilution factor, *Sample stock solution* to *Sample solution*, 9500

Acceptance criteria: 460–540 mg/100 mL of $C_8H_6N_4O_5$

PERFORMANCE TESTS

• UNIFORMITY OF DOSAGE UNITS (905)

For Oral Suspension packaged in single-unit containers: Meets the requirements

• DELIVERABLE VOLUME (698):

For Oral Suspension packaged in multiple-unit containers: Meets the requirements

IMPURITIES

• LIMIT OF *N*-(AMINOCARBONYL)-*N*-[[[5-NITRO-2-FURANYL]METHYLENE]-AMINO]GLYCINE (NF 250)

Buffer and Mobile phase: Prepare as directed in the *Assay*.

Standard solution: 2.5 μ g/mL of USP Nitrofurantoin Related Compound A RS in *Mobile phase*

Sample solution: Nominally 0.05 mg/mL of nitrofurantoin in *Mobile phase* from Oral Suspension. Centrifuge a portion of this solution. Pass a portion of the supernatant through a polytetrafluoroethylene filter having a 5- μ m pore size, discarding the first few mL.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

[NOTE—Adjust the operating parameters so that the NF 250 peak has a retention time of between 3 and 6 min and its height is about 0.1 full scale.]

Mode: LC

Detector: UV 375 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection size: 30–60 μ L

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The height of any peak appearing in the *Sample solution* at a retention time corresponding to that of the main peak from the *Standard solution* is NMT the height of the main peak from the *Standard solution* (NMT 5.0%).

SPECIFIC TESTS

• **PH (791):** 4.5–6.5

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP Nitrofurantoin RS

USP Nitrofurantoin Related Compound A RS

(*N*-(Aminocarbonyl)-*N*-[[[5-nitro-2-furanyl]-methylene]-amino]glycine).

Nitrofurantoin Tablets

DEFINITION

Nitrofurantoin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of nitrofurantoin ($C_8H_6N_4O_5$).

IDENTIFICATION

• A. INFRARED ABSORPTION

Sample: Add 10 mL of 6 N acetic acid to an amount equivalent to 100 mg of nitrofurantoin from powdered Tablets. Boil for a few min, and filter while hot. Cool to room temperature, collect the precipitate of nitrofurantoin, and dry at 105° for 1 h.

Acceptance criteria: The IR absorption spectrum of a mineral oil dispersion of the precipitate from the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Nitrofurantoin RS.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer: Dissolve 6.8 g of monobasic potassium phosphate in 500 mL of water. Add about 30 mL of 1.0 N sodium hydroxide sufficient to adjust to a pH of 7.0, and dilute with water to 1 L.

Mobile phase: Acetonitrile and *Buffer* (12:88)

Internal standard solution: 1 mg/mL of acetanilide in water

Standard solution: 0.56 mg/mL of USP Nitrofurantoin RS prepared as follows. Dissolve 50 mg of USP Nitrofurantoin RS in 40.0 mL of dimethylformamide, and add 50.0 mL of *Internal standard solution*.

Sample solution: Nominally 0.56 mg/mL of nitrofurantoin prepared as follows. Mix an amount equivalent to 50 mg of nitrofurantoin from powdered Tablets (NLT 20) with 40.0 mL of dimethylformamide, and shake mechanically for 15 min. Add 50.0 mL of *Internal standard solution*, mix, and cool to room temperature. Pass a portion of the solution through a nylon filter having a pore size of 0.45- μ m, discarding the first few mL of the filtrate.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

[NOTE—Adjust the operating parameters so that the retention time of the nitrofurantoin peak is about 8 min and the peak height is about half full-scale.]

Mode: LC**Detector:** UV 254 nm**Column:** 3.9-mm × 30-cm; packing L1**Injection size:** 5–10 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 3.0 between acetanilide and nitrofurantoin**Relative standard deviation:** NMT 2.0% determined from the ratio of the peak responses**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of nitrofurantoin (C₈H₆N₄O₅) in the portion of powdered Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = internal standard ratio (peak response of nitrofurantoin/peak response of acetanilide) from the *Sample solution*

R_S = internal standard ratio (peak response of nitrofurantoin/peak response of acetanilide) from the *Standard solution*

C_S = concentration of USP Nitrofurantoin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of nitrofurantoin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%**PERFORMANCE TESTS****• DISSOLUTION (711)****Medium:** pH 7.2 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL**Apparatus 1:** 100 rpm**Times:** 60 and 120 min**Standard stock solution:** 0.1 mg/mL of USP Nitrofurantoin RS prepared as follows. Dissolve the required amount of USP Nitrofurantoin RS in 5% of the final volume of dimethylformamide. Dilute to final volume with *Medium*.**Standard solution:** 10 µg/mL of USP Nitrofurantoin RS in *Medium* from *Standard stock solution***Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.**Analysis****Samples:** *Standard solution* and *Sample solution***Analytical wavelength:** 375 nm**Blank:** *Medium*Calculate the percentage of the labeled amount of nitrofurantoin (C₈H₆N₄O₅) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance from the *Sample solution*

A_S = absorbance from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

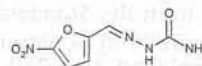
Tolerances: NLT 25% (Q) of the labeled amount of nitrofurantoin (C₈H₆N₄O₅) is dissolved in 60 min, and NLT 85% (Q) of the labeled amount of nitrofurantoin (C₈H₆N₄O₅) is dissolved in 120 min.**• UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**IMPURITIES****• LIMIT OF NITROFURAZONE****Buffer:** Proceed as directed in the *Assay*.**Mobile phase:** Tetrahydrofuran and *Buffer* (10:90)**System suitability stock solution:** 5.0 µg/mL each of USP Nitrofurazone RS and USP Nitrofurantoin RS in dimethylformamide**System suitability solution:** 0.5 µg/mL each of USP Nitrofurazone RS and USP Nitrofurantoin RS in *Mobile phase* from *System suitability stock solution***Standard stock solution:** 5.0 µg/mL of USP Nitrofurazone RS in dimethylformamide.**Standard solution:** Transfer 2.0 mL of the *Standard stock solution* into a glass-stoppered flask, and add 20.0 mL of water.**Sample solution:** Transfer an equivalent to 100 mg of nitrofurantoin from powdered Tablets into a 25-mL, glass-stoppered flask. Add 2.0 mL of dimethylformamide, and shake for 5 min. Add 20.0 mL of water, mix, and allow to stand for 15 min. Pass a portion of the mixture through a nylon filter of 0.45-µm pore size.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

[NOTE—Adjust the operating parameters so that the nitrofurazone peak has a retention time of about 10.5 min and its height is about 0.1 full-scale.]

Mode: LC**Detector:** UV 375 nm**Column:** 3.9-mm × 30-cm; packing L1**Flow rate:** 1.6 mL/min**Injection size:** 60–100 µL**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 4.0 between the nitrofurantoin and nitrofurazone peaks, *System suitability solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution***Acceptance criteria:** The height of any peak from the *Sample solution* at a retention time corresponding to that of the main peak from the *Standard solution* is NMT the height of the main peak from the *Standard solution* (NMT 0.01%).**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.**• USP REFERENCE STANDARDS (11)**

USP Nitrofurantoin RS

USP Nitrofurazone RS

NitrofurazoneC₆H₆N₄O₄ 198.14

Hydrazinecarboxamide, 2-[(5-nitro-2-furanyl)methylene]-5-Nitro-2-furaldehyde semicarbazone [59-87-0].

» Nitrofurazone, dried at 105° for 1 hour, contains not less than 98.0 percent and not more than 102.0 percent of C₆H₆N₄O₄.

NOTE—Avoid exposing solutions of nitrofurazone at all times to direct sunlight, excessive heat, strong fluorescent lighting, and alkaline materials.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

USP Reference standards (11)—

USP Nitrofurazone RS

USP Nitrofurazone Related Compound A RS

5-Nitro-2-furfuraldiazine.

$C_{10}H_6N_4O_6$ 278.18

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 8 µg per mL, prepared as directed in the Assay.

Ratio: A_{306} / A_{375} does not exceed 0.25.

C: Dissolve 400 mg of potassium hydroxide in 10 mL of alcohol. Immediately before use dilute this solution with dimethylformamide to 100 mL. To 10 mL of the prepared solution add a few crystals of Nitrofurazone: a purple solution results.

pH (791)—Suspend 1 g in 100 mL of water, shake for 15 minutes, allow the suspension to settle, and filter: the pH of the filtrate is between 5.0 and 7.5.

Loss on drying (731)—Dry it at 105° for 1 hour: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.1%.

Ordinary impurities (466)—

Test solution: dimethylformamide.

Standard solution: dimethylformamide.

Application volume: 10 µL.

Eluant: a mixture of chloroform, methanol, and ammonium hydroxide (60:24:3), in a nonequilibrated chamber.

Visualization: 1.

Limit of 5-nitro-2-furfuraldiazine—

Adsorbent: 0.5-mm layer of chromatographic silica gel.

Test solution—Transfer 2.0 g to a 100-mL volumetric flask. Dissolve in 60 mL of dimethylformamide, dilute with acetone to volume, and mix.

Standard solution—Transfer 50.0 mg of USP Nitrofurazone Related Compound A RS to a 100-mL volumetric flask, dissolve in and dilute with dimethylformamide to volume, and mix. [NOTE—USP Nitrofurazone Related Compound A RS is 5-nitro-2-furfuraldiazine.] Transfer 5.0 mL of this solution to a 25-mL volumetric flask, add 10 mL of dimethylformamide, dilute with acetone to volume, and mix.

Application volume: 5 µL.

Developing solvent system: a mixture of ethyl acetate and cyclohexane (4:1).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). With a suitable densitometer, equipped with a filter having its maximum transmittance at about 254 nm, locate and scan the spot produced by the *Standard solution* and any spot from the *Test solution* having the same R_f as that produced by the *Standard solution*: the area and intensity of any spot from the *Test solution* are not greater than the area and intensity produced by the spot from the *Standard solution* (0.5%).

Assay—Transfer about 100 mg of Nitrofurazone, previously dried and accurately weighed, to a 250-mL volumetric flask, dissolve in 50 mL of dimethylformamide, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with water to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Nitrofurazone RS in the same medium having a known concentration of about 8 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 375 nm, with a suitable spectrophotome-

ter, using water as the blank. Calculate the quantity, in mg, of $C_{10}H_6N_4O_6$ in the Nitrofurazone taken by the formula:

$$12.5C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Nitrofurazone RS in the Standard solution, and A_U and A_S are the absorbances of the solution of Nitrofurazone and the Standard solution, respectively.

Nitrofurazone Ointment

» Nitrofurazone Ointment is Nitrofurazone in a suitable water-miscible base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nitrofurazone ($C_{10}H_6N_4O_6$).

NOTE—Avoid exposure at all times to direct sunlight, excessive heat, strong fluorescent lighting, and alkaline materials.

Packaging and storage—Preserve in tight, light-resistant containers. Avoid exposure to direct sunlight, strong fluorescent lighting, and excessive heat.

USP Reference standards (11)—

USP Nitrofurazone RS

Completeness of solution—One g dissolves in 9 mL of water to form a clear solution.

Identification—Dissolve 400 mg of potassium hydroxide in a mixture of 9.5 mL of alcohol and 0.5 mL of methanol. Immediately before use, dilute with dimethylformamide to 100 mL. To 10 mL of this solution add a quantity of Ointment, equivalent to about 10 µg of nitrofurazone, and mix: a purple solution results.

Assay—[NOTE—Protect from light all solutions that contain nitrofurazone.]

Triethylamine buffer—Transfer 100 mL of triethylamine to a 1000-mL volumetric flask. Add about 800 mL of water, and cautiously add 80 mL of phosphoric acid. Mix, allow to cool to ambient temperature, dilute with water to volume, mix, and pass through a nylon filter having a 0.5-µm or finer porosity.

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and *Triethylamine buffer* (790:200:10). Make any necessary adjustments (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Transfer about 50 mg of USP Nitrofurazone RS, accurately weighed, to a 50-mL low-actinic volumetric flask, add 10 mL of dimethylformamide, and swirl to dissolve. Dilute with alcohol to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL low-actinic volumetric flask, dilute with alcohol to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL low-actinic volumetric flask containing 15 mL of alcohol, dilute with water to volume, and mix. This solution contains about 0.01 mg of USP Nitrofurazone RS per mL.

Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 1 mg of nitrofurazone, to a 100-mL low-actinic volumetric flask. Add 0.2 mL of dimethylformamide and about 25 mL of alcohol, and sonicate for about 35 minutes. Dilute with water to volume, mix, and pass through a nylon filter having a 0.5-µm or finer porosity. Use the filtrate.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 365-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as di-

rected for *Procedure*: the column efficiency determined from the nitrofurazone peak is not less than 1500 theoretical plates, and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of nitrofurazone ($C_6H_6N_4O_4$) in the portion of Ointment taken by the formula:

$$100C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Nitrofurazone RS in the *Standard preparation*; and r_u and r_s are the nitrofurazone peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nitrofurazone Topical Solution

» Nitrofurazone Topical Solution contains not less than 95.0 percent and not more than 105.0 percent (w/w) of the labeled amount of $C_6H_6N_4O_4$. **NOTE**—Avoid exposure at all times to direct sunlight, excessive heat, and alkaline materials.

Packaging and storage—Preserve in tight, light-resistant containers. Avoid exposure to direct sunlight and excessive heat.

USP Reference standards (11)—
USP Nitrofurazone RS

Identification—Dissolve 400 mg of potassium hydroxide in a mixture of 9.5 mL of alcohol and 0.5 mL of methanol. Immediately before use dilute with dimethylformamide to 100 mL. To 10 mL of this solution add 1 drop of Topical Solution: a purple solution results.

Assay—[**NOTE**—Protect from light all solutions that contain nitrofurazone.]

Triethylamine buffer, Mobile phase, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay under Nitrofurazone Ointment*.

Assay preparation—Transfer an accurately measured portion of Topical Solution, equivalent to about 1 mg of nitrofurazone, to a 100-mL low actinic volumetric flask. Add 0.2 mL of dimethylformamide and about 25 mL of warm (between 40° and 50°) alcohol. Dilute with water to volume, and mix.

Procedure—Proceed as directed in the *Assay under Nitrofurazone Ointment*. Calculate the quantity, in mg, of $C_6H_6N_4O_4$ in the portion of Topical Solution taken by the formula:

$$100C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Nitrofurazone RS in the *Standard preparation*, and r_u and r_s are the nitrofurazone peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ammonia N 13 Injection

» Ammonia N 13 Injection is a sterile solution of $^{13}NH_3$ in Sodium Chloride Injection, suitable for intravenous administration, in which a portion of

the molecules are labeled with radioactive ^{13}N (see *Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* (823)). It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{13}N expressed in MBq (or mCi) per mL at the time indicated in the labeling.

Specific activity: no carrier added.

Packaging and storage—Preserve in single-dose or multiple-dose containers that are adequately shielded.

Labeling—Label it to include the following, in addition to the information specified for *Labeling* (7), *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{13}N as ammonia expressed as total MBq (mCi) per mL, at time of calibration; the expiration time and date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations correction is to be made for radioactive decay and also indicates that the radioactive half-life of ^{13}N is 9.96 minutes. The label also includes the statement "Do not use if cloudy or if it contains particulate matter."

USP Reference standards (11)—

USP Ammonium Chloride RS

USP Endotoxin RS

Identification—

A: Radionuclidic identity—Its half-life, determined using a suitable detector system (see *Radioactivity* (821)) is between 9.5 and 10.5 minutes.

B: Radiochemical identity—The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the *Radiochemical purity* test.

Bacterial Endotoxins Test (85) (see *Sterilization and Sterility Assurance under Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses* (823))—It contains not more than 175/V USP Endotoxin Unit per mL of the Injection, in which V is the maximum administered total dose, in mL, at the expiration time.

pH (791): between 4.5 and 7.5.

Radiochemical purity—

Mobile phase—Add 0.25 mL of concentrated nitric acid to 1000 mL of a mixture of water and methanol (7:3), filter, and degas.

Standard solution—Dissolve an accurately weighed quantity of USP Ammonium Chloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg per mL.

Test solution—Use the Injection.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 4.1-mm \times 25-cm column that contains 10- μ m packing L17. It is equipped with a gamma ray detector and a conductivity detector. The flow rate is about 2.0 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5%.

Procedure—Prepare a mixture of the *Standard solution* and the *Test solution* (1:1), and inject about 20 μ L of the mixture into the chromatograph, record the chromatograms, and measure the peak areas. The areas of both the main radioactive and nonradioactive peaks are equal. [NOTE—The volume of Injection may be adjusted to obtain suitable detection system sensitivity.] The radioactivity of the major peak is not less than 95% of the total radioactivity measured. The retention time of the *Test solution* corresponds to the retention time of the *Standard solution*.

Change to read:**Radionuclidic purity** ^o(see *Radioactivity* (821)).

(CN 1-May-2017)—Using a suitable gamma-ray spectrometer

• (CN 1-May-2017), count an appropriate aliquot of the Injection for a period of time sufficient to obtain a gamma spectrum. The resultant gamma spectrum should be analyzed for the presence of identifiable photopeaks which are not characteristic of ¹³N emissions. Not less than 99.5% of the observed gamma emissions should correspond to the 0.511 MeV, 1.022 MeV, or Compton scatter peaks of ¹³N.

Chemical purity—This article may be synthesized by different methods and processes and, therefore, contains different impurities. The presence of unlabeled ingredients, reagents, and by-products specific to the process must be controlled, and their potential for physiological or pharmacological effects must be considered.

ALUMINUM (to be determined if Devarda's alloy is used to reduce ¹³N nitrate/nitrite)—

Aluminum standard solution—Transfer 35.17 mg of aluminum potassium sulfate dodecahydrate, accurately weighed, to a 1000-mL volumetric flask, and dilute with water to volume to obtain a solution having a known concentration of 2 µg of aluminum per mL.

Procedure—Pipet 10 mL of *Aluminum standard solution* into each of two 50-mL volumetric flasks. To each flask add 3 drops of methyl orange TS and 2 drops of 6 N ammonium hydroxide, then add 0.5 N hydrochloric acid, dropwise, until the solution turns red. To one flask add 25 mL of sodium thioglycolate TS, and to the other flask add 1 mL of edetate disodium TS. To each flask add 5 mL of eriochrome cyanine TS and 5 mL of acetate buffer TS, and add water to volume. Immediately determine the absorbance of the solution containing sodium thioglycolate TS at the wavelength of maximum absorbance at about 535 nm, with a suitable spectrophotometer, using the solution containing the edetate disodium TS as a blank. Repeat the procedure using two 1.0-mL aliquots of Injection. Calculate the quantity, in µg per mL, of aluminum in the Injection taken by the formula:

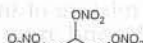
$$20(T_U / T_S)$$

in which T_U and T_S are the absorbances of the solutions from the Injection and the *Aluminum standard solution*, respectively. The concentration of aluminum ion in the Injection is not greater than 10 µg per mL.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1), except that the Injection may be distributed or dispensed prior to completion of the test for *Sterility Tests* (71), the latter test being started within 24 hours of final manufacture, and except that it is not subject to the recommendation in *Container Content*.

Assay for radioactivity—Using a suitable calibrated system as directed under *Radioactivity* (821), determine the radioactivity, in MBq (or mCi) per mL, of the Injection.

Diluted Nitroglycerin



$C_3H_5N_3O_9$ 227.09
1,2,3-Propanetriol, trinitrate;
Nitroglycerin [55-63-0].

DEFINITION

Diluted Nitroglycerin is a mixture of nitroglycerin ($C_3H_5N_3O_9$) with lactose, dextrose, alcohol, propylene glycol, or other suitable inert excipient to permit safe han-

dling. It contains NLT 90.0% and NMT 110.0% of the labeled amount of $C_3H_5N_3O_9$. It usually contains NMT 10% of nitroglycerin ($C_3H_5N_3O_9$). [**CAUTION**—Taking into consideration the concentration and amount of nitroglycerin ($C_3H_5N_3O_9$) in Diluted Nitroglycerin, exercise appropriate precautions when handling this material. Nitroglycerin is a powerful explosive and can be detonated by percussion or excessive heat. Do not isolate nitroglycerin ($C_3H_5N_3O_9$).]

IDENTIFICATION

- **A.** The R_f value of the principal spot of *Sample solution A* corresponds to that of the *Standard solution*, as obtained in the *Procedure for Organic Impurities*.
- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Mobile phase: Methanol and water (1:1)

Standard solution: 0.075 mg/mL of nitroglycerin from USP Diluted Nitroglycerin RS in *Mobile phase*

Sample solution: Transfer a portion of Diluted Nitroglycerin, equivalent to 7.5 mg of nitroglycerin, to a 100-mL volumetric flask, and dissolve in 75 mL of *Mobile phase*. If necessary, sonicate for 2 min or until the solid is totally dispersed, then shake by mechanical means for 30 min. Dilute with *Mobile phase* to volume, and filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L1. [NOTE—If necessary a short precolumn that contains packing L1 may be used.]

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 2.5 for the analyte peak

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_3H_5N_3O_9$ in the portion of Diluted Nitroglycerin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of nitroglycerin in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES**Organic Impurities**• **PROCEDURE**

Standard solution: 400 µg/mL of nitroglycerin from USP Diluted Nitroglycerin RS in methanol

Sample solution A: Prepare a clear solution containing 400 µg/mL of nitroglycerin from Diluted Nitroglycerin in methanol.

Sample solution B: 20 mg/mL of nitroglycerin in methanol from Diluted Nitroglycerin. Centrifuge a portion, if necessary, to obtain a clear liquid phase.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 μ L each of *Sample solution A* and *Sample solution B*; 5, 10, 15, and 20 μ L of the *Standard solution*

Developing solvent system: Toluene and ethyl acetate (4:1)

Spray reagent: Diphenylamine in methanol (1 in 100)

Analysis

Samples: *Standard solution*, *Sample solution A*, and *Sample solution B*

Apply the *Samples* to a suitable thin-layer chromatographic plate. Develop the chromatograms in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with *Spray reagent*, and irradiate the plate with short- and long-wavelength UV light for 15 min.

Acceptance criteria: Any spot from *Sample solution B*, other than the principal spot, is not more intense than the spot from the 20- μ L application of the *Standard solution*. Compare the intensities of any secondary spots observed from *Sample solution B* with those of the principal spots from the *Standard solution* (corresponding to 0.5%, 1.0%, 1.5%, and 2.0%, respectively): the sum of the intensities of the secondary spots from *Sample solution B* is NMT 3%. [NOTE—Nitrates of glycerin typically have R_f values of 0.21, 0.37, and 0.61 for mono-, di-, and tri-substituted glycerins, respectively.]

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and prevent exposure to excessive heat. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS (11)**
USP Diluted Nitroglycerin RS

Nitroglycerin Injection

DEFINITION

Nitroglycerin Injection is a sterile solution prepared from Diluted Nitroglycerin; the solvent may contain Alcohol, Propylene Glycol, and Water for Injection. Nitroglycerin Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$).

IDENTIFICATION

- **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Mobile phase: Methanol and water (500:500)

Standard solution: 0.075 mg/mL of nitroglycerin from USP Diluted Nitroglycerin RS in *Mobile phase*

Sample solution: Transfer a measured volume of Injection equivalent to 7.5 mg of nitroglycerin to a 100-mL volumetric flask, and dissolve in and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 25-cm; packing L1

[NOTE—If necessary, a short precolumn that contains packing L1 may be used.]

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of nitroglycerin in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

- **ALCOHOL DETERMINATION, Method II (611)**

Sample: Use isopropyl alcohol as the internal standard.

Acceptance criteria: 90.0%–110.0% of the labeled amount of C_2H_5OH

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.1 USP Endotoxin Unit/ μ g of nitroglycerin
- **PH (791)**
Sample: To 5 mL of the Injection add 5 mL of water and 1 drop of saturated potassium chloride solution.
Acceptance criteria: 3.0–6.5
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.
- **LABELING:** Where necessary, label it to indicate that it is to be diluted before use.
- **USP REFERENCE STANDARDS (11)**
USP Diluted Nitroglycerin RS
USP Endotoxin RS

Nitroglycerin Ointment

DEFINITION

Nitroglycerin Ointment is Diluted Nitroglycerin in a suitable ointment base. It contains NLT 90.0% and NMT 115.0% of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$).

IDENTIFICATION

- **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Mobile phase: Methanol and water (500:500)

Standard solution: 0.075 mg/mL of nitroglycerin from USP Diluted Nitroglycerin RS in *Mobile phase*

Sample solution: Transfer a quantity of Ointment equivalent to 2.0 mg of nitroglycerin to a glass-stoppered, 50-mL conical flask, and add 25.0 mL of *Mobile phase*. Immerse the flask containing the sample in a water bath maintained at a temperature of 50° for 10

min. Shake intermittently until the sample is dispersed. Remove the flask from the bath, and shake vigorously for 1 min to obtain a coagulated solid. Repeat the heating and shaking steps one more time, and filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L1

[NOTE—If necessary, a short precolumn that contains packing L1 may be used.]

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of nitroglycerin in the *Standard solution* (mg/mL)

C_U = nominal concentration of nitroglycerin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

- **HOMOGENEITY**

Analysis: In the case of single-dose containers, perform the Assay on specimens from each of 10 containers. In the case of multiple-dose containers, perform the Assay on one sample from the top and one from the bottom of each of five containers.

Acceptance criteria: Each sample contains NLT 90.0% and NMT 110.0% of the mean value.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label multiple-dose containers with a direction to close tightly immediately after each use.
- **USP REFERENCE STANDARDS (11)**
USP Diluted Nitroglycerin RS

Nitroglycerin Sublingual Tablets

DEFINITION

Nitroglycerin Sublingual Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

Standard solution: Equivalent to 1 mg/mL of nitroglycerin in acetone from USP Diluted Nitroglycerin RS

Sample solution: Equivalent to 1 mg/mL of nitroglycerin from powdered Sublingual Tablets, in acetone.

Shake by mechanical means for 30 min, and filter.

Developing solvent system: Toluene, ethyl acetate, and glacial acetic acid (16:4:1)

Analysis: Proceed as directed. Spray with a solution (1 in 100) of diphenylamine in methanol, and irradiate the

plate with short- and long-wavelength UV light for 10 min.

Acceptance criteria: Meets the requirements

- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Mobile phase: Methanol and water (50:50)

Standard solution: 0.075 mg/mL of nitroglycerin from USP Diluted Nitroglycerin RS in *Mobile phase*

Sample solution: Nominally equivalent to 0.075 mg/mL of nitroglycerin from powdered Sublingual Tablets (NLT 20 Sublingual Tablets) in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L1. [NOTE—If necessary, use a short precolumn that contains packing L1.]

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$) in the portion of Sublingual Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of nitroglycerin in the *Standard solution* (mg/mL)

C_U = nominal concentration of nitroglycerin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

- **DISINTEGRATION (701)**

Determined as set forth for Sublingual Tablets

Time: 2 min

Acceptance criteria: Meets the requirements

- **UNIFORMITY OF DOSAGE UNITS (905)**

Procedure for content uniformity

Mobile phase, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Sample solution: 0.075 mg/mL of nitroglycerin in *Mobile phase*, from 1 Sublingual Tablet

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$) in the Sublingual Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of nitroglycerin in the *Standard solution* (mg/mL)

C_U = nominal concentration of nitroglycerin in the *Sample solution* (mg/mL)

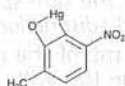
Acceptance criteria: The content of each of the 10 Sublingual Tablets is within the range of 75.0%–135.0% of the labeled claim. If the content of NMT 1 Sublingual Tablet is outside the range of 75.0%–135.0% and if the content of none of the Sub-

lingual Tablets is outside the range of 60.0%–150.0%, test 20 additional units. The requirements are met if the content of each of the additional 20 units falls within the range of 75.0%–135.0% of the labeled claim.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, preferably of glass, and store at controlled room temperature. Each container holds NMT 100 Sublingual Tablets.
- **LABELING:** The labeling indicates that the Sublingual Tablets are for sublingual use, and the label directs that the Sublingual Tablets be dispensed in the original, unopened container, labeled with the following statement directed to the patient. "Warning: To prevent loss of potency, keep these tablets in the original container or in a supplemental nitroglycerin container specifically labeled as being suitable for Nitroglycerin Sublingual Tablets. Close tightly immediately after each use."
- **USP REFERENCE STANDARDS (11)**
USP Diluted Nitroglycerin RS

Nitromersol



$C_7H_5HgNO_3$ 351.71
7-Oxa-8-mercurabicyclo[4.2.0]octa-1,3,5-triene, 5-methyl-2-nitro-;
5-Methyl-2-nitro-7-oxa-8-mercurabicyclo[4.2.0]octa-1,3,5-triene [133-58-4].

DEFINITION

Nitromersol, dried at 105° for 2 h, contains NLT 98.0% and NMT 100.5% of nitromersol ($C_7H_5HgNO_3$).

IDENTIFICATION

- **A.**
Sample solution: 1 mg/mL in 1 N sodium hydroxide
Analysis: The *Sample solution* possesses a reddish-orange color. Add 3 N hydrochloric acid.
Acceptance criteria: The reddish orange color disappears, and a yellowish, flocculent precipitate forms.
- **B.**
Sample solution: Dissolve 250 mg of Nitromersol in 2.5 mL of 1 N sodium hydroxide, and dilute with water to 20 mL.
Analysis 1: Add 3 mL of 3 N hydrochloric acid to the *Sample solution*.
Acceptance criteria 1: A yellowish precipitate is formed.
Analysis 2: Filter the solution obtained after *Analysis 1*. The filtrate is nearly colorless or slightly yellow. Retain the filtrate for the test for *Mercury Ions*. Dissolve the precipitate in 20 mL of water to which 2.5 mL of 1 N sodium hydroxide has been added. Add 0.5 g of sodium hydrosulfite, and heat to boiling.
Acceptance criteria 2: A heavy deposit of metallic mercury is formed.

ASSAY

• PROCEDURE

Sample: 200 mg of Nitromersol, previously ground to a fine powder and dried

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N ammonium thiocyanate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 500-mL Kjeldahl flask. Add 15 mL of sulfuric acid, and digest cautiously with occasional swirling over a flame until the solution becomes a clear, light yellowish brown. Cool, and add, dropwise, enough 30% hydrogen peroxide to decolorize the solution. Digest for 2–3 min, adding more hydrogen peroxide if necessary, to produce a colorless solution. Cool, dilute with water to 100 mL, and add potassium permanganate TS until a permanent pink color persists on heating. Then add hydrogen peroxide TS, dropwise, until the color is completely discharged. Cool, and add 5 mL of nitric acid that has been diluted with 10 mL of water. Add 5 mL of ferric ammonium sulfate TS, and titrate with *Titrant*. Each mL of 0.1 N ammonium thiocyanate is equivalent to 17.59 mg of nitromersol ($C_7H_5HgNO_3$).

Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

• MERCURY IONS

Sample solution: The filtrate obtained in *Identification test B, Analysis 2*

Analysis: Add an equal volume of hydrogen sulfide TS to the *Sample solution*.

Acceptance criteria: No darkening in color is produced, although a small amount of a flocculent, light yellow precipitate may form.

• ALKALI-INSOLUBLE SUBSTANCES

Sample: 1.0 g of Nitromersol

Analysis: Add 7 mL of 1 N sodium hydroxide to the *Sample*, then dilute with water to 20 mL. The resulting solution, upon standing in a glass-stoppered vessel in the dark for 24 h, shows NMT a slight amount of insoluble material. Collect the insoluble residue, if any, in a tared filter crucible, wash the residue with warm water, and dry at 105° for 1 h.

Acceptance criteria: 0.1%; the weight of the insoluble material is NMT 1 mg.

• UNCOMBINED NITROCRESOL

Sample: 500 mg of Nitromersol

Analysis: Shake the *Sample* with 50 mL of benzene, filter, evaporate the filtrate in a tared dish to dryness, and dry the residue at 80° for 2 h.

Acceptance criteria: 1%; the weight of the residue is NMT 5 mg.

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Nitromersol Topical Solution

DEFINITION

Nitromersol Topical Solution yields NLT 180.0 mg and NMT 220.0 mg of nitromersol ($C_7H_5HgNO_3$) in each 100 mL.

Nitromersol	2 g
Sodium Hydroxide	0.4 g
Sodium Carbonate, Monohydrate	4.25 g
Purified Water, a sufficient quantity to make	1000 mL

Dissolve the *Sodium Hydroxide* and the *Sodium Carbonate, Monohydrate* in 50 mL of *Purified Water*. Add the *Nitromersol*, and stir until dissolved. Gradually add *Purified Water* to make 1000 mL.

[NOTE—Prepare dilutions of *Nitromersol Topical Solution* as needed, because they tend to precipitate upon standing.]

IDENTIFICATION

- **A.**
Sample: 100 mL
Analysis: Add 3 mL of 3 N hydrochloric acid to the *Sample*.
Acceptance criteria: A yellowish precipitate is formed. Filter, and retain both the filtrate and the precipitate.
- **B.**
Sample: The precipitate obtained from *Identification test A*.
Analysis: Add the *Sample* to 20 mL of water and 2.5 mL of 1 N sodium hydroxide. Add 500 mg of sodium hydrosulfite, and heat to boiling.
Acceptance criteria: A heavy deposit of metallic mercury is formed.

ASSAY

• PROCEDURE

Sample: 50.0 mL of *Topical Solution*

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N ammonium thiocyanate VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 500-mL Kjeldahl flask, add a few glass beads, and evaporate to 5 mL. Add 15 mL of sulfuric acid, digest cautiously with occasional swirling over a flame until the solution becomes a clear, light yellowish brown. Cool, and add, dropwise, enough 30% hydrogen peroxide to decolorize the solution. Digest for 2–3 min, adding more hydrogen peroxide, if necessary, to produce a colorless solution. Cool, dilute with water to 100 mL, and add potassium permanganate TS until a permanent pink color persists on heating. Then add hydrogen peroxide TS, dropwise, until the color is completely discharged. Cool, and add 5 mL of nitric acid that has been diluted with 10 mL of water. Add 5 mL of ferric ammonium sulfate TS, and titrate with *Titrant*. Each mL of *Titrant* is equivalent to 17.59 mg of nitromersol ($C_7H_5HgNO_3$).

Acceptance criteria: 180.0–220.0 mg of nitromersol ($C_7H_5HgNO_3$) in each 100 mL

SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 1.005–1.010

• MERCURY IONS

Sample: The filtrate obtained from *Identification test A*

Analysis: To the *Sample* add an equal volume of hydrogen sulfide TS.

Acceptance criteria: No darkening in color is produced, although a small amount of a flocculent, light yellow precipitate may be formed.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers.

Nitrous Oxide

N_2O 44.01

Nitrogen oxide (N_2O).

Nitrogen oxide (N_2O) [10024-97-2].

» Nitrous Oxide contains not less than 99.0 percent, by volume, of N_2O .

Packaging and storage—Preserve in cylinders.

NOTE—The following tests are designed to reflect the quality of Nitrous Oxide in both the vapor and liquid phases that are present in previously unopened cylinders. Reduce the container pressure by means of a regulator. Withdraw the samples for the tests with the least possible release of Nitrous Oxide consistent with proper purging of the sampling apparatus. Measure the gases with a gas volume meter downstream from the detector tubes in order to minimize contamination or change of the specimens. Perform tests in the sequence in which they are listed.

The various detector tubes called for in the respective tests are listed under *Reagents* in the section *Reagents, Indicators, and Solutions*.

Identification—

A: With the container temperatures the same and maintained between 15° and 25°, concomitantly read the pressure of the Nitrous Oxide container and of a container of nitrous oxide certified standard (see under *Reagents* in the section *Reagents, Indicators, and Solutions*). [NOTE—Do not use the nitrous oxide certified standard if it has been depleted to less than half of its full capacity.] The pressure of the Nitrous Oxide container is within 50 psi of that of the nitrous oxide certified standard.

B: Pass 100 ± 5 mL released from the vapor phase of the contents of the Nitrous Oxide container through a carbon dioxide detector tube at the rate specified for the tube: no color change is observed (*distinction from carbon dioxide*).

C: Collect about 100 mL of the gas under test in a 100-mL tube fitted at the top with a stopcock. Open the stopcock, and quickly add a freshly prepared solution of 500 mg of pyrogallol in 2 mL of water and a freshly prepared solution of 12 g of potassium hydroxide in 8 mL of water. Immediately close the stopcock, and mix: the gas is not absorbed, and the solution does not become brown (*distinction from oxygen*).

Water—It meets the requirements of the test for *Water* under *Carbon Dioxide*.

Limit of ammonia—Proceed with Nitrous Oxide as directed in the test for *Carbon monoxide*, except to use an ammonia detector tube: the indicator change corresponds to not more than 0.0025%.

Limit of nitric oxide—Pass 500 ± 50 mL, released from the vapor phase of the contents of the container, through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube: the indicator change corresponds to not more than 1 ppm.

Carbon monoxide—Pass 1000 ± 50 mL, released from the vapor phase of the contents of the container, through a carbon monoxide detector tube at the rate specified for the tube: the indicator change corresponds to not more than 0.001%.

Nitrogen dioxide—Arrange a container so that when its valve is opened, a portion of the liquid phase of the contents is released through a piece of tubing of sufficient length to allow all of the liquid to vaporize during passage through it, and to prevent frost from reaching the inlet of the detector tube. Release into the tubing a flow of liquid sufficient to provide 550 mL of the vaporized sample plus any excess necessary to ensure adequate flushing of air from the system. Pass 550 ± 50 mL of this gas through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube: the indicator change corresponds to not more than 1 ppm.

Halogens—Pass 1000 ± 50 mL, released from the vapor phase of the contents of the container, through a chlorine detector tube at the rate specified for the tube: the indicator change corresponds to not more than 1 ppm.

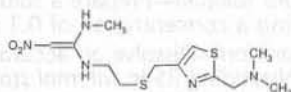
Carbon dioxide—Pass 1000 ± 50 mL, released from the vapor phase of the contents of the container, through a carbon dioxide detector tube at the rate specified for the

tube: the indicator change corresponds to not more than 0.03%.

Air—Not more than 1.0% of air is present, determined as directed in the Assay.

Assay—Introduce a specimen of Nitrous Oxide taken from the liquid phase, as directed in the test for Nitrogen dioxide, into a gas chromatograph by means of a gas-sampling valve. Select the operating conditions of the gas chromatograph such that the peak response resulting from the following procedure corresponds to not less than 70% of the full-scale reading. Preferably, use an apparatus corresponding to the general type in which the column is 6 m in length and 4 mm in inside diameter and is packed with porous polymer beads, which permits complete separation of N₂ and O₂ from N₂O, although the N₂ and O₂ may not be separated from each other. Use industrial grade helium (99.99%) as the carrier gas, with a thermal-conductivity detector, and control the column temperature: the peak response produced by the assay specimen exhibits a retention time corresponding to that produced by an air-helium certified standard (see under *Reagents* in the section *Reagents, Indicators, and Solutions*), and is equivalent to not more than 1.0% of air when compared to the peak response of the air-helium certified standard, indicating not less than 99.0%, by volume, of N₂O.

Nizatidine



C₁₂H₂₁N₅O₂S₂ 331.46

1,1-Ethenediamine, N-[2-[[[2-[(dimethylamino)methyl]-4-thiazolyl)methyl]thio]ethyl]-N'-methyl-2-nitro-N-[2-[[[2-[(Dimethylamino)methyl]-4-thiazolyl)methyl]thio]ethyl]-N'-methyl-2-nitro-1,1-ethenediamine [76963-41-2].

» Nizatidine contains not less than 98.0 percent and not more than 101.0 percent of C₁₂H₂₁N₅O₂S₂, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Nizatidine RS

Identification—

A: Infrared Absorption (197K).

B: The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that of the Standard preparation as obtained in the Assay.

Loss on drying (731)—Dry about 2 g, accurately weighed, at 100° for 1 hour: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals, Method II** (231): 0.001%. • (Official 1-Jan-2018)

Chromatographic purity—

Solution A—Use Buffer solution prepared as directed in the Assay.

Solution B—Use methanol.

Diluent—Prepare a mixture of Solution A and Solution B (76:24).

Mobile phase—Use variable mixtures of Solution A and Solution B as directed for the Chromatographic system. Make adjustments if necessary (see *System Suitability* under Chromatography (621)).

Standard solutions—Dissolve an accurately weighed quantity of USP Nizatidine RS quantitatively, and stepwise if necessary, in Diluent, sonicating if necessary, to obtain a solution having a known concentration of 50 µg per mL (Standard solution 1). Quantitatively dilute portions of Standard solution 1 with Diluent to obtain Standard solution 2 and Standard solution 3 having known concentrations of 25 µg per mL and 15 µg per mL, respectively.

Test solution—Prepare a solution of Nizatidine in Diluent having a concentration of about 5 mg per mL.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–3	76	24	isocratic
3–20	76→50	24→50	linear gradient
20–45	50	50	isocratic
45–50	50→76	50→24	linear gradient
50–70	76	24	isocratic

Make adjustments to the composition of the Mobile phase, if necessary, to obtain a retention time of about 12 minutes for the main nizatidine peak (see *System Suitability* under Chromatography (621)). Chromatograph Standard solution 1, and record the peak areas as directed for Procedure: the tailing factor is not more than 2.0.

Procedure—Separately inject equal volumes (about 50 µL) of Standard solution 1, Standard solution 2, Standard solution 3, and the Test solution into the chromatograph, and allow the Test solution to elute for not less than three times the retention time of nizatidine. Record the chromatograms, and measure the areas for all the peaks. The sum of the peak areas, excluding the nizatidine peak area, obtained from the Test solution is not more than three times the main peak area obtained from Standard solution 2; and no single peak area obtained from the Test solution is greater than the main peak area obtained from Standard solution 3; not more than 0.3% of any individual impurity is found; and not more than 1.5% of total impurities is found.

Assay—

Buffer solution—Prepare a 0.1 M solution by dissolving 5.9 g of ammonium acetate in 760 mL of water. Add 1 mL of diethylamine, and adjust with acetic acid to a pH of 7.5.

Mobile phase—Prepare a filtered and degassed mixture of Buffer solution and methanol (76:24). Make adjustments if necessary (see *System Suitability* under Chromatography (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Nizatidine RS in Mobile phase, sonicating if necessary, to obtain a solution having a known concentration of about 0.3 mg per mL.

Assay preparation—Transfer an accurately weighed quantity of 15 mg of Nizatidine to a 50-mL volumetric flask, dissolve in Mobile phase, sonicating if necessary, dilute with Mobile phase to volume, and mix.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the peak areas as directed for Procedure: the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than

2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{12}H_{21}N_5O_2S_2$ in the portion of Nizatidine taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Nizatidine RS in the *Standard preparation*; and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nizatidine Capsules

» Nizatidine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nizatidine ($C_{12}H_{21}N_5O_2S_2$).

Packaging and storage—Preserve in tight, light-resistant containers. Store at controlled room temperature.

USP Reference standards (11)—

USP Nizatidine RS

Identification—

A: Empty the contents of 2 Capsules into a beaker, add 20 mL of methanol, and swirl for approximately 2 minutes. Filter through a filter paper, and evaporate the methanol solution with a current of cool air to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Nizatidine RS.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{12}H_{21}N_5O_2S_2$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 314 nm using filtered portions of the solution under test, diluted with water if necessary, in comparison with a *Standard solution* having a known concentration of USP Nizatidine RS in the same medium.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{12}H_{21}N_5O_2S_2$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Chromatographic purity—[NOTE—Use peak areas where peak responses are indicated.]

Buffer solution and Mobile phase—Prepare as directed in the *Assay under Nizatidine*.

Standard solution—Dissolve an accurately weighed quantity of USP Nizatidine RS in *Mobile phase* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 40 μ g per mL.

Test solution—Remove as completely as possible the contents of not less than 20 Capsules, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of nizatidine, to a 100-mL volumetric flask, add 50 mL of *Mobile phase*, and sonicate for about 3 minutes. Dilute with *Mobile phase* to volume, mix, and filter.

System suitability solution—Prepare a solution of nizatidine and phenol in *Mobile phase* containing 40 μ g of each per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm \times 15-cm column that contains 5- μ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed under *Procedure*: the resolution, R , between the nizatidine and phenol peaks is not less than 1.5, the tailing factor for the nizatidine peak is not greater than 1.5, and the relative standard deviation of the nizatidine peak for replicate injections is not more than 2%.

Procedure—Chromatograph about 10 μ L of the *Standard solution* and the *Test solution*, and run the chromatograph for twice the elution time of nizatidine. Record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Capsules taken by the formula:

$$2(r_i / r_s)$$

in which r_i is the response of each impurity peak in the *Test solution*, and r_s is the response of the nizatidine peak in the *Standard solution*: not more than 0.5% of any individual impurity and not more than 1.5% of total impurities is found.

Assay—[NOTE—Use peak areas where peak responses are indicated.]

Buffer solution and Mobile phase—Prepare as directed in the *Assay under Nizatidine*.

Internal standard solution—Prepare a solution of phenol in *Mobile phase* having a concentration of 0.1 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Nizatidine RS in *Internal standard solution* to obtain a solution having a known concentration of 0.1 mg per mL.

Assay preparation—Weigh accurately not less than 10 Capsules. Remove as completely as possible the contents of the Capsules, and mix the combined contents. Clean and accurately weigh the Capsule shells, and calculate the net weight of the Capsule contents. Transfer an accurately weighed portion of the mixed Capsule contents equivalent to about 500 mg of nizatidine to a 500-mL volumetric flask, add 200 mL of *Internal standard solution*, and sonicate for a few minutes. Cool, dilute with *Internal standard solution* to volume, and mix. Filter a portion of the solution, transfer 1.0 mL of the filtered solution to a 10-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm \times 15-cm column that contains 5- μ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R , between nizatidine and the internal standard phenol, is not less than 3; the tailing factor, T , for the nizatidine peak is not more than 1.6; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 25 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for phenol and 1.0 for nizatidine. Calculate the quantity, in mg, of $C_{12}H_{21}N_5O_2S_2$ in the portion of Capsules taken by the formula:

$$5000C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Nizatidine RS in the *Standard preparation*, and R_U and R_S are the ratios of the peak response of the nizatidine to that of the internal standard for the *Assay preparation* and the *Standard preparation*, respectively.

Nonoxynol 9



α -(*p*-Nonylphenyl)- ω -hydroxynona(oxyethylene)
[26027-38-3].

DEFINITION

Nonoxynol 9 is an anhydrous liquid mixture consisting chiefly of monononylphenyl ethers of polyethylene glycols corresponding to the formula:



in which the average value of n is 9. It contains NLT 90.0% and NMT 110.0% of nonoxynol 9.

IDENTIFICATION

- **A. INFRARED ABSORPTION:** Its IR absorption spectrum, obtained by spreading a capillary film of it between sodium chloride plates, exhibits maxima at 1117 cm^{-1} (strong); at 1512, 1582, and 1610 cm^{-1} (medium, sharp); at 2871, 2928, and 2956 cm^{-1} (strong, unresolved); at 831 cm^{-1} (medium, broad); and at 1250 cm^{-1} (medium, sharp).
- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Mobile phase: Methanol and water (80:20)

System suitability solution: 25 mg/mL each of octoxynol 9 and USP Nonoxynol 9 RS in *Mobile phase*

Standard solution: 25 mg/mL of USP Nonoxynol 9 RS in *Mobile phase*

Sample solution: 25 mg/mL of Nonoxynol 9 in *Mobile phase*

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm \times 25-cm; 10- μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

[NOTE—Nonoxynol oligomers elute as a major peak, usually with shoulders and bumps. Include these in the peak response for Nonoxynol 9.]

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the responses for Nonoxynol 9, including any shoulders and bumps.

Calculate the percentage of Nonoxynol 9 in the portion of specimen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of nonoxynol 9 from the *Sample solution*

r_S = peak response of nonoxynol 9 from the *Standard solution*

C_S = concentration of USP Nonoxynol 9 RS in the *Standard solution* (mg/mL)

C_U = concentration of Nonoxynol 9 in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

FREE ETHYLENE OXIDE

Stripped nonoxynol 9: Maintain Nonoxynol 9 at a temperature of 150° with constant stirring in an open vessel until it no longer displays a peak for ethylene oxide when chromatographed as directed below.

System suitability solution: 10 $\mu\text{g}/\text{mL}$ each of ethylene oxide and acetaldehyde in *Stripped nonoxynol 9*

Standard stock solutions: [NOTE—Ethylene oxide is toxic and flammable. Prepare these solutions in a well-ventilated hood, using great care.] Chill all apparatus and reagents used in the solution of standards in a refrigerator or freezer before use. Fill a chilled pressure bottle with liquid ethylene oxide from a lecture bottle, and store in a freezer when not in use. Use a small piece of polyethylene film to protect the liquid from contact with the rubber gasket. Transfer 100 mL of chilled isopropyl alcohol to a 500-mL volumetric flask. Using a chilled graduated cylinder, transfer 25 mL of ethylene oxide to the isopropyl alcohol, and swirl gently to mix. Dilute with additional chilled isopropyl alcohol to volume, replace the stopper, and swirl gently to mix. This stock solution contains 43.6 mg/mL of ethylene oxide.

Pipet 25 mL of 0.5 N alcoholic hydrochloric acid, prepared by mixing 45 mL of hydrochloric acid with 1 L of alcohol, into a 500-mL conical flask containing 40 g of magnesium chloride hexahydrate. Shake the mixture to effect saturation. Pipet 10 mL of the ethylene oxide solution into the flask, and add 20 drops of bromocresol green TS. If the solution is not yellow (acid), add an additional volume of 0.5 N alcoholic hydrochloric acid to give an excess of 10 mL. Record the total volume of 0.5 N alcoholic hydrochloric acid added. Insert the stopper in the flask, and allow to stand for 30 min. Titrate the excess acid with 0.5 N alcoholic potassium hydroxide VS. Perform a blank titration, using 10.0 mL of isopropyl alcohol instead of ethylene oxide solution, adding the same total volume of 0.5 N alcoholic hydrochloric acid, and note the difference in volumes required. Each mL of the difference in volumes of 0.5 N alcoholic potassium hydroxide consumed is equivalent to 22.02 mg of ethylene oxide. Calculate the concentration, in mg/mL, of ethylene oxide in the stock solution. Standardize daily. Store in a refrigerator.

Prepare a 1000-ppm standard by pipeting the calculated volume (2 mL) of cold stock solution that, on the basis of the standardization, contains 88.6 mg of ethylene oxide, into a container and adding 87.0 g of *Stripped nonoxynol 9*. Prepare 10-, 5-, and 0.5-ppm standards by quantitatively diluting the 1000-ppm standard with additional *Stripped nonoxynol 9*.

Standard solutions: Transfer 5 ± 0.01 g of each *Standard stock solution* to suitable serum vials equipped with pressure-tight septum closures designed to relieve any excessive pressure, and seal them.

Sample solution: Transfer 5 ± 0.01 g of Nonoxynol 9 to a serum vial of the same kind as the vials used for the *Standard solutions*.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 6.4-m \times 2.1-mm nickel; packed with 60- to 80-mesh support S9

Temperatures

Detector: 200°

Injection port: 160°

Column: 100°

Carrier gas: Helium

Flow rate: 30 mL/min

Injection volume: 100 µL

System suitability**Samples:** *System suitability solution* and *Standard solutions*

Calibration: Place the vial containing the 10-ppm ethylene oxide *Standard solution* in an oven, and heat at 90° for 30 min. Remove the vial from the oven. Using a gas-tight syringe, immediately inject a 100-µL aliquot of the headspace gas into the gas chromatograph. Obtain the area for the ethylene oxide peak (retention time about 8 min). Raise the temperature of the column to 200° after ethylene oxide elutes to volatilize heavy components. Re-equilibrate the column at 100°. Repeat the foregoing steps, using the vials containing the 5- and 0.5-ppm *Standard solution*. Plot area units versus ppm of ethylene oxide for the standards on linear graph paper, and draw the best straight line through the points.

Suitability requirements**Resolution:** NLT 1.5 between ethylene oxide and acetaldehyde, *System suitability solution***Calibration:** None of the points used for constructing the straight line *Calibration* curve deviates from the line by more than 10%.**Analysis****Samples:** *Standard solutions* and *Sample solution*

Place the vial containing the *Sample solution* in an oven, and heat at 90° for 30 min. Remove the vial from the oven. Immediately inject a 100-µL aliquot of the headspace gas into the gas chromatograph, and obtain the area for the ethylene oxide peak.

Calculate the concentration of ethylene oxide in the sample specimen in ppm:

$$\text{Result} = r_U \times S$$

 r_U = peak area from the *Sample solution* S = slope of the standard curve (ppm/area unit)**Acceptance criteria:** NMT 1 ppm**• LIMIT OF DIOXANE**

Apparatus: Assemble a closed-system vacuum distillation apparatus (see Figure 1), using glass vacuum stopcocks (A, B, and C). The concentrator tube (D)¹ is made of borosilicate or quartz (not flint) glass, graduated precisely enough to measure the 0.9 mL or more of distillate collected, and marked so that the analyst can dilute accurately to 2.0 mL.

Standard solution: 100 µg/mL of dioxane in water. Use a freshly prepared solution.

Sample solution: Transfer 20.0 g to a 50-mL round-bottom flask (E) having a 24/40 ground-glass neck joint. Add 1.0 mL of water. Place a small polytetrafluoroethylene-covered stirring bar in the flask, insert the stopper, and stir to mix. Immerse the flask in an ice bath, and chill for 1 min. Wrap heating tape around the tube connecting the concentrator tube (D) and the round-bottom flask, and apply 10 V to the tape. Apply a light coating of high-vacuum silicone grease to the ground-glass joints, and connect the concentrator tube to the 10/30 joint and the round-bottom flask to the 24/40 joint. Immerse the vacuum trap in a Dewar flask filled with liquid nitrogen,

close stopcocks A and B, open stopcock C, and begin evacuating the system with a vacuum pump. Prepare a slurry bath from powdered dry ice and methanol, and raise the bath to the neck of the round-bottom flask. After freezing the contents of the flask for 10 min, and when the vacuum system is operating at a 0.05-mm pressure or lower, open stopcock A for 20 s, then close it. Remove the slurry bath, and allow the flask to warm in air for 1 min. Immerse the flask in a water bath maintained at a temperature between 20° and 25°, and after 5 min warm the water bath to between 35° and 40° (sufficient to liquefy most specimens) while stirring slowly but constantly with the magnetic bar. Cool the water in the bath by adding ice, and chill for 2 min. Replace the water bath with the slurry bath, freeze the contents of the round-bottom flask for 10 min, open stopcock A for 20 s, and then close it. Remove the slurry bath, and repeat the heating steps as before, this time reaching a final temperature between 45° and 50° or a temperature necessary to melt the specimen completely. If there is any condensation in the tube connecting the round-bottom flask to the concentrator tube, slowly increase the voltage to the heating tape, and heat until condensation disappears.

Stir with the magnetic stirrer throughout the following steps. Very slowly immerse the concentrator tube in a Dewar flask containing liquid nitrogen. **[CAUTION—**When there is liquid distillate in the concentrator tube, immerse the tube in the liquid nitrogen very slowly, or the tube will break.] Water will begin to distill into the concentrator tube. As ice forms in the concentrator tube, raise the Dewar flask to keep the liquid nitrogen level only slightly below the level of ice in the tube. When water begins to freeze in the neck of the 10/30 joint, or when liquid nitrogen reaches the 2.0-mL graduation mark on the concentrator tube, remove the Dewar flask, and allow the ice to melt without heating. After the ice has melted, check the volume of water that has distilled, and repeat the sequence of chilling and thawing until NLT 0.9 mL of water has been collected. Freeze the tube once again for 2 min, and release the vacuum first by opening stopcock B, followed by opening stopcock A. Remove the concentrator tube from the apparatus, close it with a greased stopper, and allow the ice to melt without heating. Mix the contents of the concentrator tube by swirling, note the volume of distillate, and dilute with water to 2.0 mL, if necessary.

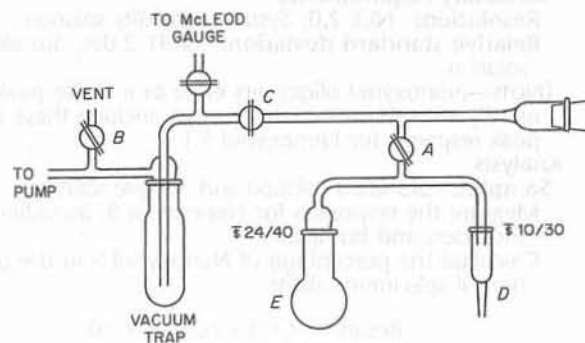


Figure 1. Closed-System Vacuum Distillation Apparatus for Dioxane

¹ A suitable tube is available as Chromaflex concentrator tube, Kontes Glass Co., Vineland, NJ (Catalog No. K42560-0000).

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m glass; contains support S10

Temperatures

Detector: 250°

Injection port: 200°

Column: 140°

Carrier gas: Nitrogen or helium

Flow rate: 35 mL/min

Injection volume: 2–4 µL

Install an oxygen scrubber between the carrier gas line and the column. Condition the column for 72 h at 230° with 30–40 mL/min carrier flow. [NOTE—Support S10 is oxygen sensitive. Each time a column is installed, flush with carrier gas for 30–60 min before heating.]

AnalysisSamples: *Standard solution* and *Sample solution*

Acceptance criteria: The height of the peak of the *Sample solution* is NMT that of the *Standard solution*, corresponding to NMT 10 µg/g.

SPECIFIC TESTS• **POLYETHYLENE GLYCOL**

Sample: 10 g

Analysis: Transfer the *Sample* to a 250-mL beaker. Add 100 mL of ethyl acetate, and stir on a magnetic stirrer to effect solution. Transfer, with the aid of 100 mL of 5 N sodium chloride, to a pear-shaped, 500-mL separator fitted with a glass stopper. Insert the stopper, and shake vigorously for 1 min. Remove the stopper carefully to release the pressure. Immerse a thermometer in the mixture, and support the separator so that it is partially immersed in a water bath maintained at 50°. Swirl the separator gently while letting the internal temperature rise to between 40° and 45°, then immediately remove the separator from the bath, dry the outside surface, and drain the salt (lower) layer into another pear-shaped, 500-mL separator. In the same manner, extract the ethyl acetate layer a second time with 100 mL of fresh 5 N sodium chloride, combining the two aqueous extracts. Discard the ethyl acetate layer. Wash the combined aqueous layers with 100 mL of ethyl acetate, using the same technique, and drain the salt (lower) layer into a clean pear-shaped, 500-mL separator. Discard the ethyl acetate layer. Extract the aqueous layer with two successive 100-mL portions of chloroform, draining the chloroform (lower) layers through Whatman folded filter paper 2V, and combining them into a 250-mL beaker. Evaporate on a steam bath to dryness, and continue heating until the odor of chloroform is no longer perceptible. Allow the beaker to cool. Add 25 mL of acetone, and dissolve the residue on a magnetic stirrer. Filter through Whatman folded filter paper 2V into a tared 250-mL beaker, rinsing with two 25-mL portions of acetone. Evaporate on a steam bath to dryness. Dry under vacuum at 60° for 1 h. Allow the beaker to cool, and weigh.

Acceptance criteria: NMT 1.0% of polyethylene glycol

• **CLOUD POINT**

Sample: 1.0 g

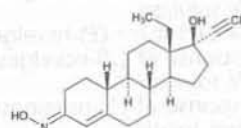
Analysis: Transfer the *Sample* to a 250-mL beaker, add 99 g of water, and mix to dissolve. Pour 30 mL of the resulting solution into a 70-mL test tube. Support the test tube in a hot water bath, and stir the contents with a thermometer constantly until the solution becomes cloudy, then remove the test tube from the bath immediately so that the temperature rises NMT 2° further, and continue stirring. The cloud point is the temperature at which the solution becomes sufficiently clear that the entire thermometer bulb is seen plainly.

Acceptance criteria: 52°–56°

- **FATS AND FIXED OILS**, *Acid Value* (401): NMT 0.2
- **WATER DETERMINATION**, *Method I* (921): NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)
USP Nonoxynol 9 RS

Norelgestromin

$C_{21}H_{29}NO_2$ 327.46
18,19-Dinorpregn-4-en-20-yn-3-one, 13-ethyl-17-hydroxy-, oxime, (17 α)-;
13-Ethyl-17-hydroxy-18,19-dinor-17 α -pregn-4-en-20-yn-3-one oxime [53016-31-2].

DEFINITION

Norelgestromin is a mixture of (*E*)- and (*Z*)-isomers having a ratio of (*E*)- to (*Z*)-isomer between 1.3 and 1.6, and the sum of both isomers is NLT 98.0% and NMT 102.0% of norelgestromin ($C_{21}H_{29}NO_2$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY• **PROCEDURE**

Mobile phase: Cyclohexane and absolute alcohol (100:2)

Diluent: Cyclohexane and absolute alcohol (90:10)

System suitability solution: 1.5 mg/mL of USP Norelgestromin RS and 8 µg/mL of USP Norelgestromin Related Compound A RS in *Diluent*

Standard solution: 1.5 mg/mL of USP Norelgestromin RS in *Diluent*

Sample solution: 1.5 mg/mL of Norelgestromin in *Diluent*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L20

Column temperature: 50°

Flow rate: 1.2 mL/min

Injection volume: 25 µL

Run time: 1.6 times the retention time of (*Z*)-norelgestromin

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for the (*Z*)-isomer of norelgestromin related compound A, (*E*)-norelgestromin, and (*Z*)-norelgestromin are about 0.77, 0.85, and 1.00, respectively.]

Suitability requirements

Resolution: NLT 1.4 between the (*Z*)-isomer of norelgestromin related compound A and (*E*)-norelgestromin, *System suitability solution*

Tailing factor: 0.8–1.2 for both (E)- and (Z)-norelgestromin, *System suitability solution* and *Standard solution*

Relative standard deviation: NMT 0.73% for both (E)- and (Z)-norelgestromin, *System suitability solution* and *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of norelgestromin ($C_{21}H_{29}NO_2$) in the portion of Norelgestromin taken:

$$\text{Result} = \{[(r_{UE} \times F) + r_{UZ}]/[(r_{SE} \times F) + r_{SZ}]\} \times (C_S/C_U) \times 100$$

r_{UE} = peak response of (E)-norelgestromin from the *Sample solution*

F = response factor for (E)-norelgestromin, 1.04

r_{UZ} = peak response of (Z)-norelgestromin from the *Sample solution*

r_{SE} = peak response of (E)-norelgestromin from the *Standard solution*

r_{SZ} = peak response of (Z)-norelgestromin from the *Standard solution*

C_S = concentration of USP Norelgestromin RS in the *Standard solution* (mg/mL)

C_U = concentration of Norelgestromin in the *Sample solution* (mg/mL)

Calculate the ratio of (E)- to (Z)-norelgestromin:

$$\text{Result} = (r_{UE} \times F)/r_{UZ}$$

r_{UE} = peak response of (E)-norelgestromin from the *Sample solution*

F = response factor for (E)-norelgestromin, 1.04

r_{UZ} = peak response of (Z)-norelgestromin from the *Sample solution*

Acceptance criteria

Both isomers: 98.0%–102.0% on the anhydrous basis
Ratio of (E)- to (Z)-isomer: 1.3–1.6

IMPURITIES

• **RESIDUE ON IGNITION (281):** NMT 0.2%

• ORGANIC IMPURITIES

Mobile phase, Diluent, *System suitability solution*, *Standard solution*, *Sample solution*, and *Chromatographic system*: Proceed as directed in the *Assay*.

Sensitivity solution: 1.5 µg/mL of USP Norelgestromin RS in *Diluent* from the *Standard solution*

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.4 between the (Z)-isomer of norelgestromin related compound A and (E)-norelgestromin, *System suitability solution*

Tailing factor: 0.8–1.2 for both (E)- and (Z)-norelgestromin, *System suitability solution* and *Standard solution*

Relative standard deviation: NMT 0.73% for both (E)- and (Z)-norelgestromin, *System suitability solution* and *Standard solution*

Signal-to-noise ratio: NLT 3 for both (E)- and (Z)-norelgestromin, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Norelgestromin taken:

$$\text{Result} = \{r_U/[(r_{SE} \times F) + r_{SZ}]\} \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_{SE} = peak response of (E)-norelgestromin from the *Standard solution*

F = response factor for (E)-norelgestromin, 1.04

r_{SZ} = peak response of (Z)-norelgestromin from the *Standard solution*

C_S = concentration of USP Norelgestromin RS in the *Standard solution* (mg/mL)

C_U = concentration of Norelgestromin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard peaks that are less than 0.05% of the total peak areas of (E)- and (Z)-norelgestromin.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Norgestrel ^a	0.37	0.5
(E)- and (Z)-Norgestimate ^b	0.43 ^c , 0.47 ^d	0.5 ^e
Norelgestromin 5(10)-ene ^{f,g}	0.68	—
Norelgestromin related compound A ^g	0.73 ^h , 0.77 ⁱ	—
(E)-Norelgestromin	0.85	—
(Z)-Norelgestromin	1.0	—
Any other individual impurity	—	0.10
Total impurities	—	1.0

^a (±)-13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one.

^b 18,19-Dinor-17-pregn-4-en-20-yn-3-one, 17-(acetyloxy)-13-ethyl-oxime, (17α)-(+)-; (+)-13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one oxime acetate (ester).

^c (E)-Norgestimate.

^d (Z)-Norgestimate.

^e The combined limits for (E)- and (Z)-norgestimate are NMT 0.5%.

^f 13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-5(10)-en-20-yn-3-one oxime.

^g This is not a specified impurity and is included in this table for identification only. It is not to be reported or included in the total impurities.

^h (E)-Isomer of norelgestromin related compound A.

ⁱ (Z)-Isomer of norelgestromin related compound A.

SPECIFIC TESTS

• OPTICAL ROTATION, *Specific Rotation* (781S)

Sample solution: 5 mg/mL in alcohol and water (75:25)

Acceptance criteria: +35° to +41°

• WATER DETERMINATION, *Method 1c* (921): NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Norelgestromin RS

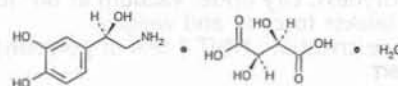
USP Norelgestromin Related Compound A RS

Mixture of (E)- and (Z)-isomers.

13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-5(6)-en-20-yn-3-one oxime.

$C_{21}H_{29}NO_2$ 327.46

Norepinephrine Bitartrate



$C_8H_{11}NO_3 \cdot C_4H_6O_6 \cdot H_2O$ 337.28

$C_8H_{11}NO_2 \cdot C_4H_6O_6$ 319.27

1,2-Benzenediol, 4-(2-amino-1-hydroxyethyl)-, (R)-, [R-(R*, R*)]-2,3-dihydroxybutanedioate (1:1) (salt), monohydrate; (–)-α-(Aminomethyl)-3,4-dihydroxybenzyl alcohol tartrate (1:1) (salt), monohydrate [108341-18-0].

Anhydrous [51-40-1].

DEFINITION

Norepinephrine Bitartrate contains NLT 97.0% and NMT 102.0% of $C_8H_{11}NO_3 \cdot C_4H_6O_6$, calculated on the anhydrous basis.

IDENTIFICATION• **A. INFRARED ABSORPTION** (197K)• **B. PROCEDURE**

Sample solution: 5 mg/mL in water

Analysis: Add 1 drop of ferric chloride TS.

Acceptance criteria: An intensely green color develops.

• **C. PROCEDURE**

Sample solution: 0.01 µg/mL

Analysis: Add 1.0 mL of 0.10 N iodine to 10 mL of the Sample solution. Allow to stand for 5 min, and add 2.0 mL of 0.10 N sodium thiosulfate.

Acceptance criteria: The solution is colorless, or has at most a slight pink or slight violet color (epinephrine and isoproterenol at the same pH, about 3.5, give a strong red-brown or violet color).

ASSAY• **PROCEDURE**

Sample solution: 25 mg/mL of Norepinephrine Bitartrate in glacial acetic acid. If necessary warm slightly to effect solution.

Analysis: Add 2 drops of crystal violet TS to the Sample solution, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 31.93 mg of $C_8H_{11}NO_3 \cdot C_4H_6O_6$.

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES**Inorganic Impurities**• **RESIDUE ON IGNITION** (281): NMT 0.1%, from 200 mg**Organic Impurities**• **PROCEDURE: LIMIT OF ARTERENONE**

Sample solution: 2 mg/mL in water

Analysis: Determine the absorptivity of the Sample solution (see *Ultraviolet-Visible Spectroscopy* (857)) at 310 nm.

Acceptance criteria: NMT 0.2

SPECIFIC TESTS• **OPTICAL ROTATION, Specific Rotation** (781S): -10° to -12°

Sample solution: 50 mg/mL in water

• **WATER DETERMINATION, Method I** (921): 4.5%–5.8%**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.• **USP REFERENCE STANDARDS** (11)

USP Norepinephrine Bitartrate RS

Norepinephrine Bitartrate Injection

» Norepinephrine Bitartrate Injection is a sterile solution of Norepinephrine Bitartrate in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of norepinephrine ($C_8H_{11}NO_3$).

Packaging and storage—Preserve in single-dose, light-resistant containers, preferably of Type I glass.

Labeling—Label the Injection in terms of mg of norepinephrine per mL, and, where necessary, label it to indicate that it must be diluted prior to use. The label indicates that

the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

USP Reference standards (11)—

USP Endotoxin RS

USP Norepinephrine Bitartrate RS

Color and clarity—

Standard solution—Transfer 2.0 mL of 0.100 N iodine VS to a 500-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Visually examine a portion of the Injection (*Test solution*) in a suitable clear glass test tube against a white background: it is not pinkish and it contains no precipitate. If any yellow color is observed in the *Test solution*, concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells with a suitable spectrophotometer set at 460 nm: the absorbance of the *Test solution* does not exceed that of the *Standard solution*.

Identification—

A: It responds to Identification test B under Norepinephrine Bitartrate.

B: Dilute the Injection with water to a concentration of 1 mg in 5 mL. To 10 mL of the dilution add 2.0 mL of 0.10 N iodine, allow to stand for 5 minutes, then add 3.0 mL of 0.10 N sodium thiosulfate: the solution is colorless or has at most a slight pink or slight violet color (epinephrine and isoproterenol at the same pH, about 3.5, give a red-brown or violet color).

Bacterial Endotoxins Test (85)—It contains not more than 83.4 USP Endotoxin Units per mg of norepinephrine.

pH (791): between 3.0 and 4.5.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Mobile phase—Dissolve 1.1 g of sodium 1-heptanesulfonate in 800 mL of water. Add 200 mL of methanol, and adjust with 1 M phosphoric acid to a pH of 3.0 ± 0.1 . Pass through a membrane filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Norepinephrine Bitartrate RS in freshly prepared dilute acetic acid (1 in 25), and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.4 mg of norepinephrine bitartrate monohydrate per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of norepinephrine, to a 25-mL volumetric flask, add dilute acetic acid (1 in 25) to volume, and mix.

System suitability preparation—Dissolve a suitable quantity of isoproterenol hydrochloride in the *Standard preparation* to obtain a solution containing, in each mL, 0.4 mg of USP Norepinephrine Bitartrate RS and 0.4 mg of isoproterenol hydrochloride.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm \times 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed under *Procedure*: the tailing factor for the analyte peak is not more than 2.5, the resolution, *R*, between the norepinephrine and isoproterenol peaks is not less than 4.0, and the relative standard deviation for replicate injections is not more than 2.0%.

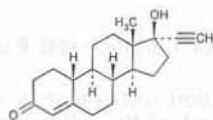
Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of norepinephrine ($C_8H_{11}NO_3$) in each mL of the Injection taken by the formula:

$$(169.18 / 337.29)(25C / V)(r_U / r_S)$$

in which 169.18 and 337.29 are the molecular weights of norepinephrine and norepinephrine bitartrate monohydrate, respectively; C is the concentration, in mg per mL, of USP Norepinephrine Bitartrate RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Norethindrone



$C_{20}H_{26}O_2$ 298.42

19-Norpregn-4-en-20-yn-3-one, 17-hydroxy-, (17 α)-
17-Hydroxy-19-nor-17 α -pregn-4-en-20-yn-3-one
[68-22-4].

» Norethindrone contains not less than 97.0 percent and not more than 102.0 percent of $C_{20}H_{26}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Norethindrone RS

Completeness of solution—The solution called for in the test for *Specific rotation* is clear and free from undissolved solid.

Identification, Infrared Absorption (197K).

Melting range (741): between 202° and 208°.

Specific rotation (781S): between −30° and −38°.

Test solution: 20 mg per mL, in dioxane.

Loss on drying (731)—Dry it in vacuum at 105° for 3 hours: it loses not more than 0.5% of its weight.

Limit of ethynyl group—Dissolve 200 mg in about 40 mL of tetrahydrofuran. Add 10 mL of silver nitrate solution (1 in 10), and titrate with 0.1 N sodium hydroxide VS, either a glass-calomel or a silver-silver chloride electrode system with potassium nitrate filling solution. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium hydroxide is equivalent to 2.503 mg of ethynyl group ($-C\equiv CH$). Not less than 8.18% and not more than 8.43% of ethynyl group is found.

Chromatographic purity—

Test solution—Prepare a solution of Norethindrone in chloroform to contain 10 mg per mL.

Standard solutions—Prepare a solution of USP Norethindrone RS in chloroform to contain 10 mg per mL (*Standard stock solution*). Dilute accurately measured volumes of *Standard stock solution* with chloroform to obtain *Standard solutions A, B, C, and D* having known concentrations of 150, 50, 30, and 10 μ g per mL, respectively.

Procedure—Separately apply 10 μ L of the *Test solution* and 10 μ L of each *Standard solution* to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of chloroform and methanol (95:5) until the solvent front has moved about three-fourths of the length of the

plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of methanol and sulfuric acid (7:3), then heat the plate at 100° for 5 minutes: the R_f value of the principal spot from the *Test solution* corresponds to that of the principal spot from *Standard solution A*. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solution*: no secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot obtained from *Standard solution B* (0.5%), and the sum of the intensities of the secondary spots obtained from the *Test solution* is not more intense than the principal spot obtained from *Standard solution A* (1.5%).

Assay—Dissolve about 100 mg of Norethindrone, accurately weighed, in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a solution containing about 10 μ g per mL. Dissolve an accurately weighed quantity of USP Norethindrone RS in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a *Standard solution* having a known concentration of about 10 μ g per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 240 nm, using alcohol as the blank. Calculate the quantity, in mg, of $C_{20}H_{26}O_2$ in the portion of Norethindrone taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in μ g per mL, of USP Norethindrone RS in the *Standard solution*, and A_U and A_S are the absorbances of the solution of Norethindrone and the *Standard solution*, respectively.

Norethindrone Tablets

DEFINITION

Norethindrone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of norethindrone ($C_{20}H_{26}O_2$).

IDENTIFICATION

• A. INFRARED ABSORPTION

Analysis: Mix an amount of powdered Tablets equivalent to 50 mg of norethindrone with 15 mL of solvent hexane. Stir the solution occasionally for 15 min. Centrifuge the mixture, then decant and discard the solvent hexane. Extract the residue with two 10-mL portions of solvent hexane, centrifuging and decanting as before, and discard the solvent hexane. Add 25 mL of chloroform to the residue, mix by shaking for 1–2 min, and filter. Evaporate the filtrate to about 3 mL, add a few mL of solvent hexane to induce crystallization, and evaporate to dryness.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion prepared from the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Norethindrone RS.

ASSAY

• PROCEDURE

Solution A: Dissolve 1.0 g of isoniazid in 1000 mL of anhydrous methanol, and add 1.3 mL of hydrochloric acid.

Standard stock solution: 14 μ g/mL of USP Norethindrone RS in methanol

Standard solution: Transfer 10.0 mL of the *Standard stock solution* to a suitable container, add 2.0 mL of *Solution A*, mix, seal, and allow to stand for 30 min.

Sample stock solution: Transfer an amount of finely powdered Tablets equivalent to 0.7 mg of norethindrone (powder NLT 20 Tablets) to a 50-mL volumetric flask, and add anhydrous methanol to volume. Mix, and allow to stand for 10 min, with occasional mixing. Filter a portion of the mixture to clarify the solution. Use the filtrate.

Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a suitable container. Add 2.0 mL of *Solution A*, mix, seal, and allow to stand for 30 min.

Sample blank solution: Transfer 10.0 mL of the *Sample stock solution* to a suitable container, add 2.0 mL of methanol, and mix.

Reagent blank solution: Transfer 10.0 mL of methanol to a suitable container, add 2.0 mL of *Solution A*, mix, seal, and allow to stand for 30 min.

Instrumental conditions

Mode: UV

Analytical wavelength: 380 nm

Analysis

Samples: *Standard solution*, *Sample solution*, *Sample blank solution*, and *Reagent blank solution*

Concomitantly determine the absorbances of these solutions, using methanol as the reference for the *Sample blank solution*, and using the *Reagent blank solution* as the reference for the *Sample solution* and the *Standard solution*.

Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) in the portion of Tablets taken:

$$\text{Result} = (A_U - A_{UB})/A_S \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_{UB} = absorbance of the *Sample blank solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Norethindrone RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of norethindrone in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Medium: 0.09% Sodium lauryl sulfate in 0.1 N hydrochloric acid; 500 mL, deaerated

Apparatus 2: 75 rpm

Time: 30 min

Mobile phase: Acetonitrile and water (2:3)

Standard stock solution: 0.07 mg/mL of USP Norethindrone RS in methanol. Sonication may be used to aid dissolution.

Standard solution: ($L/500$) mg/mL of USP Norethindrone RS in *Medium* from the *Standard stock solution*, where L is the claim in mg/Tablet of norethindrone

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 15-cm; 5- μm packing L7

Flow rate: 1.5 mL/min

Injection volume: 100 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of norethindrone in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.09% Sodium lauryl sulfate in 0.1 N hydrochloric acid; 500 mL, deaerated

Apparatus 2: 75 rpm

Time: 45 min

Mobile phase: Acetonitrile and 0.02 M phosphate buffer pH 6.0 (35:65)

Standard stock solution: 0.028 mg/mL of USP Norethindrone RS in methanol

Standard solution: ($L/500$) mg/mL of USP Norethindrone RS in *Medium* from the *Standard stock solution*, where L is the claim in mg/Tablet of norethindrone

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μm pore size or centrifuge at least 10 mL of the solution under test and use the supernatant.

Use one of the following two chromatographic systems.

(See *Chromatography* (621), *System Suitability*.)

Chromatographic system 1

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm \times 10-cm; 3- μm packing L1

Flow rate: 1 mL/min

Injection volume: 100 μL

Chromatographic system 2

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm \times 10-cm; 3- or 3.5- μm packing L1

Flow rate: 2 mL/min

Injection volume: 100 μL

System suitability

Use this *System suitability* for either *Chromatographic system 1* or *Chromatographic system 2*.

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of norethindrone in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: 0.5% Sodium lauryl sulfate in 0.1 N hydrochloric acid; 500 mL, deaerated

Apparatus 2: 75 rpm

Time: 20 min

Mobile phase: Acetonitrile and water (50:50)

Standard stock solution: 0.028 mg/mL of USP Norethindrone RS in methanol. Sonication may be used to aid dissolution.

Standard solution: ($L/500$) mg/mL of USP Norethindrone RS in *Medium* from the *Standard stock solution*, where L is the claim in mg/Tablet of norethindrone

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size or centrifuge and use the supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Column temperature: 30°

Flow rate: 1.3 mL/min

Injection volume: 100 μ L

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of norethindrone in the Standard solution (mg/mL)

V = volume of Medium, 500 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of norethindrone ($C_{20}H_{26}O_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)
USP Norethindrone RS

Norethindrone and Ethinyl Estradiol Tablets

» Norethindrone and Ethinyl Estradiol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norethindrone ($C_{20}H_{26}O_2$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Ethinyl Estradiol RS

USP Norethindrone RS

Identification—Crush 1 Tablet in 1 mL of alcohol in a 15-mL conical centrifuge tube, and warm to 50° for 10 minutes with gentle swirling. Cool, and centrifuge to obtain a clear solution. Apply 20 μ L of this test solution and 20 μ L of an alcohol solution containing, in each mL, about 1 mg of USP Norethindrone RS and about 50 μ g of USP Ethinyl Estradiol RS at equidistant points along a line about 2.5 cm from the bottom of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel and previously activated by heat-

ing at 105° for 30 minutes. Develop the chromatogram in a mixture of toluene and ethyl acetate (4:1) in a suitable chamber, previously equilibrated with the solvent mixture, until the solvent front has moved about 10 cm. Remove the plate, and air-dry. Spray the plate with sulfuric acid-methanol, prepared by cautiously adding 70 mL of sulfuric acid in small increments to 30 mL of methanol chilled in an ice-bath, and mixing; the spots from the test solution have the same R_f values as the spots from USP Ethinyl Estradiol RS and from USP Norethindrone RS.

Dissolution (711)—[NOTE—Exercise care in filtering and handling solutions containing ethinyl estradiol to prevent adsorptive loss of the drug. Centrifugation may be used instead of filtration with nonadsorptive membrane filters. Withdraw dissolution aliquots with glass or polytet pipets or syringes that have been checked for adsorptive loss. Use glass dissolution vessels and polytet-coated or solid polytet paddles.]

Medium: 0.09% sodium dodecyl sulfate in 0.1 N hydrochloric acid; 500 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Determine the amounts of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved, employing the following method.

Mobile phase—Prepare a degassed and filtered mixture of 0.02 M pH 6.0 phosphate buffer and acetonitrile (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 200-nm detector, and a 5-mm \times 8.3-cm column that contains 3- μ m packing L1. The flow rate is about 1 mL per minute. Chromatograph replicate injections of a filtered portion of a Standard solution of USP Norethindrone RS and USP Ethinyl Estradiol RS in *Dissolution Medium* having concentrations similar to those expected in the solution under test. [NOTE—A volume of methanol not exceeding 4% of the total final volume of the Standard solution may be used in preparing the Standard solution.] Record the peak responses as directed for *Procedure*: the resolution, R , for norethindrone and ethinyl estradiol is not less than 1.5; the minimum number of theoretical plates for the ethinyl estradiol peak is not less than 7000; the tailing factor does not exceed 2.0 for either peak; and the relative standard deviation is not more than 3.0%.

Procedure—Separately inject equal volumes (about 100 μ L) of the Standard solution and a filtered portion of the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.9 for norethindrone and 1.0 for ethinyl estradiol. Calculate the quantities of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved by comparison of the corresponding peak responses obtained from the Standard solution and the solution under test.

Tolerances—Not less than 75% (Q) of each of the labeled amounts of $C_{20}H_{26}O_2$ and $C_{20}H_{24}O_2$, respectively, are dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements for *Content Uniformity* with respect to norethindrone and to ethinyl estradiol.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard solution—Transfer about 15 mg of valerophenone into a 250-mL volumetric flask, add 125 mL of acetonitrile, dilute with water to volume, and mix.

Ethinyl estradiol standard stock solution—Dissolve an accurately weighed quantity of USP Ethinyl Estradiol RS in acetonitrile, and dilute quantitatively and stepwise with acetonitrile.

trile to obtain a solution having a known concentration of about 0.09 mg per mL.

Norethindrone standard stock solution—Using an accurately weighed quantity of USP Norethindrone RS, prepare a solution in acetonitrile having a known concentration of about 1.25 mg per mL.

Mixed standard preparation—Transfer 5.0 mL of *Internal standard solution* into a 100-mL volumetric flask. Add accurately measured volumes of *Ethinyl estradiol standard stock solution* and *Norethindrone standard stock solution* so that the final known concentrations, in mg per mL, of the Reference Standards correspond numerically to about one-twentieth of the labeled amounts of the corresponding ingredients in the Tablets. Add $(26 - X)$ mL of acetonitrile, X being the total volume of the *standard stock solution* taken. Dilute with a mixture of acetonitrile and water (45 in 100) to volume, and mix.

Assay preparation—Transfer 10 Tablets to a 100-mL volumetric flask, add 20 mL of water, and shake by mechanical means until the tablets are completely disintegrated. Add 10.0 mL of *Internal standard solution* and 60 mL of acetonitrile, and mix. Sonicate for about 2 minutes. Dilute with acetonitrile to volume, and mix. Allow solid particles to settle, or centrifuge if necessary to obtain a slightly turbid solution. Dilute 5.0 mL of this solution with a mixture of acetonitrile and water (45 in 100) to 10.0 mL, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 200-nm detector and a 4.6-mm \times 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R , between the norethindrone and ethinyl estradiol peaks is not less than 2.0; the column efficiency determined from the internal standard peak is not less than 8000 theoretical plates; and the relative standard deviation for six replicate injections is not more than 2.0% (both peaks).

Procedure—Separately inject equal volumes (about 25 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.9 for ethinyl estradiol and 1.0 for norethindrone. Calculate the quantities, in mg, of norethindrone ($C_{20}H_{26}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) in each Tablet taken by the formula:

$$20C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Mixed standard preparation*, and R_U and R_S are the peak response ratios, at corresponding retention times, obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Norethindrone and Mestranol Tablets

» Norethindrone and Mestranol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norethindrone ($C_{20}H_{26}O_2$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of mestranol ($C_{21}H_{26}O_2$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Mestranol RS

USP Norethindrone RS

Identification—Crush 1 Tablet in 1 mL of alcohol in a 15-mL conical centrifuge tube, and centrifuge briefly. Apply 10 μ L of this test solution and 10 μ L each of solutions containing, respectively, about 1 mg per mL of USP Norethindrone RS in alcohol and about 50 μ g per mL of USP Mestranol RS in alcohol at equidistant points along a line about 2.5 cm from the bottom of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel and previously activated by heating at 105° for 30 minutes. Develop the chromatogram in a mixture of equal volumes of ethyl acetate and cyclohexane in a suitable chamber, previously equilibrated with the solvent mixture, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, air-dry, and observe under short-wavelength UV light: the principal spot from the test solution appears at the same R_f value as the principal spot from USP Norethindrone RS, at about R_f 0.6. Spray the plate with a sulfuric acid and methanol mixture prepared by cautiously adding and mixing sulfuric acid in small increments to 30 mL of chilled anhydrous methanol in a 100-mL volumetric flask. Adjust to room temperature, dilute with sulfuric acid to volume, and mix. Heat the plate at 105° for 10 minutes: the pink spot from the test solution appears at the same R_f value as the pink spot from USP Mestranol RS (about R_f 0.8).

Dissolution (711)—[NOTE—Exercise care in filtering solutions containing mestranol to prevent adsorptive loss of the drug. Centrifugation may be used instead of filtration with nonadsorptive membrane filters. Withdraw dissolution aliquots with glass or polytetrafluoroethylene pipets or syringes that have been checked for adsorptive loss. Use glass dissolution vessels and polytetrafluoroethylene-coated or solid polytetrafluoroethylene paddles.]

Medium: 0.09% sodium lauryl sulfate in 0.1 N hydrochloric acid; 500 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Determine the amounts of norethindrone ($C_{20}H_{26}O_2$) and mestranol ($C_{21}H_{26}O_2$) dissolved, employing the following method.

Mobile phase—Prepare a degassed and filtered mixture of water and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm \times 25-cm column that contains packing L10. The flow rate is about 1 mL per minute. Chromatograph replicate injections of a filtered portion of a *Standard solution* of USP Norethindrone RS and USP Mestranol RS in *Dissolution Medium* having known concentrations similar to those expected in the solution under test, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 3.0%. The minimum number of theoretical plates for the mestranol peak is 4000, and the tailing factors for the norethindrone and mestranol peaks do not exceed 1.5.

Procedure—Separately inject equal volumes (about 200 μ L) of the *Standard solution* and a filtered portion of the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.4 for norethindrone and 1.0 for mestranol. Calculate the quantities of norethindrone and mestranol dissolved by comparison of the corresponding peak responses obtained from the *Standard solution* and the test solutions.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{20}H_{26}O_2$ and 75% (Q) of the labeled amount of $C_{21}H_{26}O_2$ are dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements for *Content Uniformity* with respect to norethindrone and to mestranol.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard solution—Transfer about 80 mg of progesterone into a 100-mL volumetric flask, add 50 mL of acetonitrile, dilute with water to volume, and mix.

Mestranol standard stock solution—Dissolve an accurately weighed quantity of USP Mestranol RS in acetonitrile, and dilute quantitatively and stepwise with acetonitrile to obtain a solution having a known concentration of about 0.05 mg per mL.

Norethindrone standard stock solution—Using an accurately weighed quantity of USP Norethindrone RS, prepare a solution in acetonitrile having a known concentration of about 1 mg per mL.

Standard preparation—Transfer 2.0 mL of *Internal standard solution* into a 100-mL volumetric flask. Add accurately measured volumes of *Mestranol standard stock solution* and *Norethindrone standard stock solution* so that the final known concentrations, in mg per mL, of the Reference Standards correspond numerically to about one-fiftieth of the labeled amounts of the corresponding ingredients in the Tablets. Add 50 mL of water, dilute with acetonitrile to volume, and mix.

Assay preparation—Transfer 10 Tablets to a 250-mL volumetric flask, add 50 mL of water, and shake by mechanical means until the Tablets are completely disintegrated. Add 10.0 mL of *Internal standard solution* and 165 mL of acetonitrile, and mix. Sonicate for about 2 minutes. Dilute with acetonitrile to volume, and mix. Allow solid particles to settle, or centrifuge if necessary, to obtain a slightly turbid solution. Transfer 5.0 mL of this solution to a 10-mL volumetric flask, add 1.0 mL of acetonitrile, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 200-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the mestranol peak is not less than 6000 theoretical plates, the resolution, R , between the progesterone and mestranol peaks is not less than 5.0, and the relative standard deviation for six replicate injections is not more than 2.0% (both peaks).

Procedure—Separately inject equal volumes (about 25 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 2.5 for mestranol and 1.0 for norethindrone. Calculate the quantities, in mg, of norethindrone ($C_{20}H_{26}O_2$) and mestranol ($C_{21}H_{26}O_2$) in each Tablet taken by the formula:

$$50C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*, and R_U and R_S are the peak response ratios, at corresponding retention times, obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Norethindrone Acetate

$C_{22}H_{28}O_3$ 340.46

19-Norpregn-4-en-20-yn-3-one, 17-(acetyloxy)-, (17 α).

17-Hydroxy-19-nor-17 α -pregn-4-en-20-yn-3-one acetate [51-98-9].

» Norethindrone Acetate contains not less than 97.0 percent and not more than 103.0 percent of $C_{22}H_{28}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Norethindrone Acetate RS

Completeness of solution—The solution prepared for the determination of *Specific rotation* is clear and free from undissolved solids.

Identification, Infrared Absorption (197K).

Specific rotation (781S): between -32° and -38° .

Test solution: 20 mg per mL, in dioxane.

Loss on drying (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Limit of ethynyl group—Proceed as directed in the test for *Ethynyl group* under *Norethindrone*. Not less than 7.13% and not more than 7.57% of ethynyl group is found.

Chromatographic purity—

TEST 1—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Test solution—Prepare a solution of Norethindrone Acetate in chloroform having a concentration of 10 mg per mL.

Standard stock solution—Prepare a solution of USP Norethindrone Acetate RS in chloroform having a known concentration of 10 mg per mL.

Standard solutions—Dilute accurately measured volumes of the *Standard stock solution* with chloroform to obtain *Standard solutions A, B, C, and D* having known concentrations of 150 μ g per mL, 50 μ g per mL, 30 μ g per mL, and 10 μ g per mL, respectively.

Application volume: 10 μ L, as two 5- μ L portions.

Developing solvent system: a mixture of toluene and ethyl acetate (1:1).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621), except to apply the solutions along a line 2.5 cm from the edge of the plate.

Spray the plate with a mixture of methanol and sulfuric acid (7:3), and heat at 100° for 5 minutes. The *Test solution* exhibits a principal spot at the same R_f value as the principal spot of *Standard solution A*. Any individual secondary spot is not more intense than the spot in the chromatogram obtained from *Standard solution B*: not more than 0.5% of any individual impurity is found, and the total of impurities found is not more than 1.5%.

TEST 2—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (6:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Resolution solution—Dissolve accurately weighed quantities of desoxycorticosterone acetate and USP Norethindrone Acetate RS in *Mobile phase* to obtain a solution having concentrations of about 80 μ g of each per mL.

Test solution—Transfer about 62.5 mg of Norethindrone Acetate, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Diluted test solution—Transfer 1.0 mL of the *Test solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.83 for desoxycorticosterone acetate and 1.0 for norethindrone acetate; and the resolution, R , between desoxycor-

ticosterone acetate and norethindrone acetate is not less than 3.5.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Diluted test solution* and the *Test solution* into the chromatograph, record the chromatograms for twice the retention time of norethindrone acetate, and measure all of the peak areas. Calculate the percentage of each impurity in the portion of Norethindrone Acetate taken by the formula:

$$r_i / r_s$$

in which r_i is the peak area for each impurity obtained from the *Test solution*; and r_s is the sum of all the peaks obtained from the *Diluted test solution*. [NOTE—Exclude any peak having a response that is less than 0.025%.] Not more than 0.5% of any individual impurity is found; and not more than 1.0% of total impurities is found.

Assay—Transfer about 100 mg of Norethindrone Acetate, accurately weighed, to a 200-mL volumetric flask, add alcohol to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with alcohol to volume, and mix. Dissolve an accurately weighed quantity of USP Norethindrone Acetate RS in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a Standard solution having a known concentration of about 10 μ g per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 240 nm, with a suitable spectrophotometer, using alcohol as the blank. Calculate the quantity, in mg, of $C_{22}H_{28}O_3$ in the portion of Norethindrone Acetate taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in μ g per mL, of USP Norethindrone Acetate RS in the Standard solution, and A_U and A_S are the absorbances of the solution of Norethindrone Acetate and the Standard solution, respectively.

Norethindrone Acetate Tablets

» Norethindrone Acetate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Norethindrone Acetate RS

Identification—It responds to the *Identification* test under *Norethindrone Tablets*, USP Norethindrone Acetate RS being used to prepare the Standard preparation.

Dissolution (711)—

Medium: dilute hydrochloric acid (1 in 100) containing 0.02% of sodium lauryl sulfate; 900 mL.

Apparatus 1: 100 rpm.

Time: 60 minutes.

Procedure—Determine the amount of $C_{22}H_{28}O_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 248 nm, measured from a baseline drawn from 350 nm through 310 nm and extending beyond the peak maximum, of filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a Standard solution having a known concentration of USP Norethindrone Acetate RS in the same medium. [NOTE—The Standard solution may be prepared by dissolving the Reference Standard in a volume of methanol, not exceeding

0.5% of the final volume of the solution, and diluting quantitatively with *Dissolution Medium*.]

Tolerances—Not less than 70% (Q) of the labeled amount of $C_{22}H_{28}O_3$ is dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements.

Procedure for content uniformity—Transfer 1 finely powdered Tablet to a 100-mL volumetric flask with the aid of about 75 mL of alcohol. Heat the alcohol to boiling, and allow the mixture to remain at a temperature just below the boiling point for about 15 minutes, with occasional swirling. Cool to room temperature, dilute with alcohol to volume, mix, and centrifuge a portion of the contents at about 2000 rpm until the solution becomes clear. Dilute a portion of the supernatant quantitatively and stepwise with alcohol to obtain a solution containing approximately 10 μ g of norethindrone acetate per mL. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Norethindrone Acetate RS in alcohol having a known concentration of about 10 μ g per mL in 1-cm cells at the wavelength of maximum absorbance at about 240 nm, with a suitable spectrophotometer, using alcohol as the blank. Calculate the quantity, in mg, of $C_{22}H_{28}O_3$ in the Tablet taken by the formula:

$$(TC / D)(A_U / A_S)$$

in which T is the labeled quantity, in mg, of norethindrone acetate in the Tablet, C is the concentration, in μ g per mL, of USP Norethindrone Acetate RS in the Standard solution, D is the concentration, in μ g per mL, of the solution from the Tablet, based upon the labeled quantity per Tablet and the extent of dilution, and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of norethindrone acetate, to a separator, add 10 mL of water, and extract with three 25-mL portions of chloroform, filtering each extract through chloroform-washed cotton. Evaporate the combined chloroform extracts on a steam bath to dryness, reducing the heat as dryness is approached. Dissolve the residue in alcohol, transfer the solution to a 100-mL volumetric flask, dilute with alcohol to volume, and mix. Transfer a 5.0-mL aliquot to a 100-mL volumetric flask, dilute with alcohol to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Norethindrone Acetate RS in alcohol having a known concentration of about 10 μ g per mL in 1-cm cells at the wavelength of maximum absorbance at about 240 nm, with a suitable spectrophotometer, using alcohol as the blank. Calculate the quantity, in mg, of $C_{22}H_{28}O_3$ in the portion of Tablets taken by the formula:

$$2C(A_U / A_S)$$

in which C is the concentration, in μ g per mL, of USP Norethindrone Acetate RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Tablets and the Standard solution, respectively.

Norethindrone Acetate and Ethinyl Estradiol Tablets

DEFINITION

Norethindrone Acetate and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$), and NLT 88.0% and NMT 112.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

A. The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

ASSAY**PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (40:5:55)

Diluent: Acetonitrile and water (1:1)

Standard solution: 90 µg/mL of USP Norethindrone Acetate RS and 1.76 µg/mL of USP Ethinyl Estradiol RS in *Diluent*

Sample solution: Transfer an appropriate number of Tablets, NLT 20, to a volumetric flask, such that the final concentration is 100 µg/mL of norethindrone acetate based on the label claim. Fill the flask with *Diluent* to about 75% of volume, and disintegrate the Tablets by mechanical shaking and sonication. Allow the solution to equilibrate to room temperature, and dilute with *Diluent* to volume. Centrifuge a portion of the solution using glass centrifuge tubes, and use the clear supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 100 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol and norethindrone acetate are about 0.28 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 10.0 between ethinyl estradiol and norethindrone acetate

Tailing factor: NMT 2.0 for ethinyl estradiol and norethindrone acetate

Relative standard deviation: NMT 2.0% for ethinyl estradiol and norethindrone acetate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of norethindrone acetate (C₂₂H₂₈O₃) and ethinyl estradiol (C₂₀H₂₄O₂) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding analyte from the *Sample solution*

r_S = peak response of the corresponding analyte from the *Standard solution*

C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (µg/mL)

C_U = nominal concentration of the corresponding analyte in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of norethindrone acetate (C₂₂H₂₈O₃); 88.0%–112.0% of the labeled amount of ethinyl estradiol (C₂₀H₂₄O₂)

PERFORMANCE TESTS**DISSOLUTION (711)**

0.025 M acetate buffer solution: Transfer 5.22 g of anhydrous sodium acetate and 2.2 g of glacial acetic acid to a 4-L volumetric flask, add 3.5 L of water, and adjust with 1 N sodium hydroxide to a pH of 5.0 ± 0.2. Dilute with water to volume.

Medium: 0.025 M acetate buffer solution with 0.15% sodium lauryl sulfate, prepared by weighing 6 g of sodium lauryl sulfate into a 4-L volumetric flask, adding

1.5 L of 0.025 M acetate buffer solution, mixing, and diluting with 0.025 M acetate buffer solution to volume; 600 mL

Apparatus 2: 75 rpm

Time: 60 min

Mobile phase: 0.2% phosphoric acid, acetonitrile, and tetrahydrofuran (540:380:80)

Standard solution: USP Norethindrone Acetate RS and USP Ethinyl Estradiol RS dissolved in a minimum amount of acetonitrile, and diluted with *Medium* to obtain a solution having known concentrations equivalent to the expected concentrations of the solution under test

Sample solution: Withdraw a 2-mL aliquot, using a glass pipet or syringe, and centrifuge at about 2000 rpm for about 5 min. Use the supernatant as the *Sample solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 242 nm detector and a fluorescence detector with an excitation wavelength set at 210 nm and an emission wavelength set at 310 nm

Column: 6-mm × 40-mm; 3-µm packing L1

Guard column: 4-mm × 12.5-mm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 200 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 500 theoretical plates for ethinyl estradiol and NLT 1400 theoretical plates for norethindrone acetate

Tailing factor: NMT 2.0 for norethindrone acetate and ethinyl estradiol

Relative standard deviation: NMT 2.5% for norethindrone acetate and ethinyl estradiol

Analysis

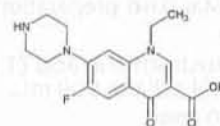
Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 80% (Q) of the labeled amounts of norethindrone acetate (C₂₂H₂₈O₃) and ethinyl estradiol (C₂₀H₂₄O₂) is dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
USP Ethinyl Estradiol RS
USP Norethindrone Acetate RS

Norfloxacin

C₁₆H₁₈FN₃O₃ 319.33
3-Quinolincarboxylic acid, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-;
1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolincarboxylic acid [70458-96-7].

DEFINITION

Norfloxacin contains NLT 98.0% and NMT 102.0% of norfloxacin (C₁₆H₁₈FN₃O₃), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: Water adjusted with phosphoric acid to a pH of 2.0

Diluent: Acetonitrile and *Solution A* (5:95)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Acetonitrile (%)
0	95	5
5	95	5
7	93	7
10	87	13
15	47	53
20	10	90
20.1	95	5
25	95	5

Standard solution: 0.1 mg/mL of USP Norfloxacin RS in *Diluent*. Sonicate, if necessary, to dissolve.

Sample solution: 0.1 mg/mL of Norfloxacin in *Diluent*. Sonicate, if necessary, to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV

Analytical wavelength: 265 nm

Column: 4.6-mm × 25-cm; 5-μm packing L60

Column temperature: 60°

Flow rate: 1.4 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 0.73%

Tailing factor: NMT 2.0

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of norfloxacin (C₁₆H₁₈FN₃O₃) in the portion of Norfloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Norfloxacin RS in the *Standard solution* (mg/mL)

C_U = concentration of Norfloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES• **RESIDUE ON IGNITION (281)**

Analysis: Use a platinum crucible.

Acceptance criteria: NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 15 ppm (Official 1: Jan-2018)

• **ORGANIC IMPURITIES**

Solution A, Diluent, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability stock solution: 0.01 mg/mL each of USP Norfloxacin Related Compound K RS, USP Norflox-

acin Related Compound E RS, USP Norfloxacin Related Compound A RS, and USP Norfloxacin Related Compound H RS in *Diluent* prepared as follows. Dissolve 1 mg each of USP Norfloxacin Related Compound K RS, USP Norfloxacin Related Compound E RS, USP Norfloxacin Related Compound A RS, and USP Norfloxacin Related Compound H RS in 10 mL of acetonitrile in a 100-mL volumetric flask. Add about 70 mL of *Diluent*. Sonicate, if necessary, to dissolve. Dilute with *Diluent* to volume.

System suitability solution: To 1 mg of USP Norfloxacin RS in a 10-mL volumetric flask add 5 mL of *System suitability stock solution*. Sonicate, if necessary, to dissolve. Dilute with *Diluent* to volume.

Standard solution: 0.4 μg/mL of USP Norfloxacin RS in *Diluent*

Sample solution: 0.4 mg/mL of Norfloxacin in *Diluent*. Sonicate, if necessary, to dissolve.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between norfloxacin related compound E and norfloxacin; NLT 3.0 between norfloxacin related compound A and norfloxacin related compound H, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Norfloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Norfloxacin RS in the *Standard solution* (μg/mL)

C_U = concentration of Norfloxacin in the *Sample solution* (mg/mL)

F = conversion factor, 0.001 mg/μg

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time (min)	Acceptance Criteria, NMT (%)
Norfloxacin related compound K	0.85	0.15
Norfloxacin related compound E	0.95	0.15
Norfloxacin	1.0	—
Norfloxacin related compound A	1.4	0.10
Norfloxacin related compound H	1.43	0.10
Unidentified impurity	1.0	0.10
Total impurities	1.0	0.5

SPECIFIC TESTS• **LOSS ON DRYING (731)**

Analysis: Dry at 100° to constant weight.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

- USP Norfloxacin RS
 USP Norfloxacin Related Compound A RS
 7-Chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.
 $C_{17}H_{18}ClFN_3O_3$ 269.66
 USP Norfloxacin Related Compound E RS
 7-Chloro-1-ethyl-4-oxo-6-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.
 $C_{16}H_{18}ClN_3O_3$ 335.79
 USP Norfloxacin Related Compound H RS
 7-[4-(Ethoxycarbonyl)piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.
 $C_{19}H_{22}FN_3O_5$ 391.39
 USP Norfloxacin Related Compound K RS
 6-Fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.
 $C_{15}H_{16}FN_3O_3$ 305.30

Norfloxacin Ophthalmic Solution

» Norfloxacin Ophthalmic Solution is a sterile, aqueous solution of Norfloxacin. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norfloxacin ($C_{16}H_{18}FN_3O_3$).

Packaging and storage—Preserve in tight, light-resistant containers, stored at controlled room temperature.

USP Reference standards (11)—

USP Norfloxacin RS

Identification—

A: Ultraviolet Absorption (197U)—

Solution: about 0.06 mg of norfloxacin per mL.

Diluent: 0.1 N hydrochloric acid.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Sterility Tests (71): meets the requirements.

pH (791): between 5.0 and 5.4.

Assay—

Dilute phosphoric acid solution—Prepare a solution of phosphoric acid in water (1 in 1000).

Mobile phase—Prepare a filtered and degassed mixture of *Dilute phosphoric acid solution* and acetonitrile (850:150). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Prepare a solution of USP Norfloxacin RS in *Dilute phosphoric acid solution* having a known concentration of about 0.06 mg per mL.

Resolution solution—Prepare a solution of USP Norfloxacin RS and pipemidic acid in *Dilute phosphoric acid solution* having known concentrations of about 0.06 mg of each per mL.

Assay preparation—Dilute an accurately measured volume of Ophthalmic Solution quantitatively and stepwise with *Dilute phosphoric acid solution* to obtain a solution having a concentration of about 0.06 mg of norfloxacin per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 278-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The column temperature is maintained at 50°. The flow rate is about 0.5 mL per minute. Precondition the column for about 8 hours with 0.01 M monobasic sodium phosphate buffer adjusted with phosphoric acid to a pH of 4.0. Chromatograph the *Resolution solution*, and record the peak re-

sponses as directed for *Procedure*: the relative retention times are about 0.8 for pipemidic acid and 1.0 for norfloxacin; and the resolution, *R*, between the pipemidic acid peak and the norfloxacin peak is not less than 1.2. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the norfloxacin peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of norfloxacin ($C_{16}H_{18}FN_3O_3$) in each mL of the Ophthalmic Solution taken by the formula:

$$(L/D)(C)(r_u/r_s)$$

in which *L* is the labeled quantity, in mg per mL, of norfloxacin in the Ophthalmic Solution; *D* is the concentration, in mg per mL, of norfloxacin in the *Assay preparation*, based on the labeled quantity of norfloxacin in each mL of the Ophthalmic Solution and the extent of dilution; *C* is the concentration, in mg per mL, of USP Norfloxacin RS in the *Standard preparation*; and *r_u* and *r_s* are the norfloxacin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Norfloxacin Tablets

» Norfloxacin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norfloxacin ($C_{16}H_{18}FN_3O_3$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Norfloxacin RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay*.

B: Shake a quantity of finely powdered Tablets, equivalent to about 75 mg of norfloxacin, with 50 mL of a mixture of acidic methanol (prepared by mixing 1000 mL of methanol and 9 mL of hydrochloric acid) and methylene chloride (1:1). Centrifuge a portion of the suspension thus obtained, and use the clear supernatant as the test solution. Apply 50 µL each of the test solution and a standard solution of USP Norfloxacin RS in the same solvent containing 1.5 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a suitable chromatographic chamber that contains and has been equilibrated with a developing system consisting of a mixture of chloroform, methanol, toluene, diethylamine, and water (40:40:20:14:8), and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the *R_f* value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Dissolution (711)—

pH 4.0 buffer—To 900 mL of water in a 1000-mL volumetric flask add 2.86 mL of glacial acetic acid and 1.0 mL of a 50% (w/w) solution of sodium hydroxide, dilute with

water to volume, and mix. If necessary, adjust with glacial acetic acid or the sodium hydroxide solution to a pH of 4.0.

Medium: pH 4.0 buffer; 750 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{16}H_{18}FN_3O_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 278 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Norfloxacin RS in the same medium.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{16}H_{18}FN_3O_3$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of phosphoric acid solution (1 in 1000) and acetonitrile (850:150). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Norfloxacin RS quantitatively in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of norfloxacin, to a 200-mL volumetric flask. Add 80 mL of *Mobile phase*, sonicate for 10 minutes, dilute with phosphoric acid solution (1 in 1000) to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and filter through a filter having a porosity of 1 μ m or less.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 275-nm detector and a 3.9-mm \times 30-cm column that contains packing L1, and is operated at $40 \pm 1.0^\circ$.

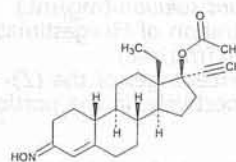
Precondition the column with degassed 0.01 M monobasic sodium phosphate adjusted with phosphoric acid to a pH of 4.0, flowing at a rate of 0.5 mL per minute for 8 hours. For the assay, use a *Mobile phase* flow rate of about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*; the capacity factor, k' , is not less than 2, the column efficiency is not less than 1500 theoretical plates, the tailing factor for the norfloxacin peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the major peaks. Calculate the quantity, in mg, of $C_{16}H_{18}FN_3O_3$ in the portion of Tablets taken by the formula:

$$500C(r_u/r_s)$$

in which C is the concentration, in mg per mL, of USP Norfloxacin RS in the *Standard preparation*, and r_u and r_s are the norfloxacin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Norgestimate



$C_{23}H_{31}NO_3$ 369.50
18,19-Dinor-17-pregn-4-en-20-yn-3-one, 17-(acetyloxy)-13-ethyl-, oxime, (17 α)-(+)-;
(+)-13-Ethyl-17-hydroxy-18,19-dinor-17 α -pregn-4-en-20-yn-3-one oxime acetate (ester) [35189-28-7].

DEFINITION

Norgestimate is a mixture of (E)- and (Z)-isomers having a ratio of (E)- to (Z)-isomer between 1.27 and 1.78, and it contains NLT 98.0% and NMT 102.0% of norgestimate ($C_{23}H_{31}NO_3$), calculated on the dried basis.

IDENTIFICATION

A. INFRARED ABSORPTION (197K)

Sample: Use a dispersion prepared by mixing with potassium bromide in a 1-to-100 ratio.

Acceptance criteria: Meets the requirements

- B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

DILUENT

Diluent: Methanol and water (4:1)

Mobile phase: Tetrahydrofuran, acetonitrile, and water (22:18:60)

Standard solution: 0.5 mg/mL of USP Norgestimate RS in *Diluent*

Sensitivity solution: 0.05 μ g/mL of USP Norgestimate RS in *Diluent*, from the *Standard solution*

Sample solution: 0.5 mg/mL of Norgestimate in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 244 nm

Column: 4.6-mm \times 10-cm; 3- μ m packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 25 μ L

System suitability

Samples: *Standard solution* and *Sensitivity solution*
[NOTE—The relative retention times for (Z)-norgestimate and (E)-norgestimate are about 0.86 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between (Z)-norgestimate and (E)-norgestimate, *Standard solution*

Tailing factor: NMT 1.5 for (E)-norgestimate and for (Z)-norgestimate, *Standard solution*

Relative standard deviation: NMT 2.0% for the peak area ratio of (E)-norgestimate to (Z)-norgestimate, *Standard solution*

Signal-to-noise ratio: NLT 3.0 for (Z)-norgestimate, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of norgestimate ($C_{23}H_{31}NO_3$) in the portion of Norgestimate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = sum of the peak areas of (Z)-norgestimate and (E)-norgestimate from the *Sample solution*

- r_s = sum of the peak areas of (Z)-norgestimate and (E)-norgestimate from the *Standard solution*
 C_s = concentration of USP Norgestimate RS in the *Standard solution* (mg/mL)
 C_u = concentration of Norgestimate in the *Sample solution* (mg/mL)
 Calculate the percentages of the (Z)- and (E)-isomers, U_z and U_e , respectively, in the portion of Norgestimate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times 100$$

- r_u = peak response of the appropriate norgestimate isomer from the *Sample solution*
 r_s = peak response of the appropriate norgestimate isomer from the *Standard solution*
 C_s = concentration of USP Norgestimate RS in the *Standard solution* (mg/mL)
 C_u = concentration of Norgestimate in the *Sample solution* (mg/mL)
 P = fraction of (E)- or (Z)-norgestimate in USP Norgestimate RS
 Calculate the ratio of U_e to U_z .
Acceptance criteria: 98.0%–102.0% of norgestimate ($C_{23}H_{31}NO_3$) on the dried basis; the ratio of (E)- to (Z)-isomer is 1.27–1.78.

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.3%

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-Jan-2018)

• ORGANIC IMPURITIES, PROCEDURE 1

Diluent, Mobile phase, Standard solution, Sensitivity solution, and Sample solution: Proceed as directed in the *Assay*.

System suitability solution: 0.5 mg/mL each of USP Norgestimate RS, USP Norgestimate Related Compound A RS, and USP Deacetylnorgestimate RS in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 244 nm

Column: 4.6-mm × 10-cm; 3-μm packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 25 μL

System suitability

Samples: *Sensitivity solution* and *System suitability solution*

[NOTE—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between (Z)-17-deacetylnorgestimate and (E)-17-deacetylnorgestimate; NLT 1.5 between (E)-17-deacetylnorgestimate and norgestimate related compound A, *System suitability solution*

Tailing factor: NMT 1.5 for (E)-norgestimate and for (Z)-norgestimate, *System suitability solution*

Relative standard deviation: NMT 2.0% for the peak area ratio of (E)-norgestimate to (Z)-norgestimate, *System suitability solution*

Signal-to-noise ratio: NLT 3.0 for (Z)-norgestimate, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Norgestimate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times P \times 100$$

r_u = peak area for each impurity from the *Sample solution*

- r_s = peak area of (E)-norgestimate from the *Standard solution*
 C_s = concentration of USP Norgestimate RS in the *Standard solution* (mg/mL)
 C_u = concentration of Norgestimate in the *Sample solution* (mg/mL)
 F = relative response factor for each impurity (see *Table 1*)
 P = fraction of (E)-norgestimate in USP Norgestimate RS

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
(Z)-17-Deacetylnorgestimate ^a	0.50	0.83	0.3
(E)-17-Deacetylnorgestimate ^a	0.56	1.13	0.3
Norgestimate related compound A ^b	0.72	0.85	0.3
(Z)-Norgestimate	0.86	—	—
(E)-Norgestimate	1.0	—	—
Any other impurity	—	1.0	0.1

^a The combined limits for (Z)- and (E)-17-deacetylnorgestimate are NMT 0.3%.

^b Levonorgestrel acetate.

• ORGANIC IMPURITIES, PROCEDURE 2

Mobile phase: Cyclohexane and absolute alcohol (50:1)

Standard solution: 1.0 mg/mL of USP Norgestimate RS in *Mobile phase*

Sensitivity solution: 0.5 μg/mL of USP Norgestimate RS in *Mobile phase* from the *Standard solution*

Sample solution: 1.0 mg/mL of Norgestimate in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-μm packing L20

Flow rate: 1 mL/min

Injection volume: 25 μL

System suitability

Samples: *Standard solution* and *Sensitivity solution*

[NOTE—The retention time for (E)-norgestimate is about 18.6 min; see *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between (Z)-norgestimate and (E)-norgestimate, *Standard solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, for the peak area ratio of (Z)-norgestimate to (E)-norgestimate, *Standard solution*

Signal-to-noise ratio: NLT 3.0 for (E)-norgestimate, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Norgestimate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times P \times 100$$

r_u = peak area for each impurity from the *Sample solution*

r_s = peak area of (E)-norgestimate from the *Standard solution*

C_s = concentration of USP Norgestimate RS in the *Standard solution* (mg/mL)

- C_U = concentration of Norgestimate in the *Sample solution* (mg/mL)
 F = relative response factor for each impurity (see Table 2)
 P = fraction of (*E*)-norgestimate in USP Norgestimate RS
Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Unidentified impurity	0.74	1.4	0.2
Unidentified impurity	0.78	1.5	0.1
Unidentified impurity	0.91	1.2	0.1
(<i>E</i>)-Norgestimate	1.0	—	—
(<i>Z</i>)-Norgestimate	1.1	—	—
Total impurities ^a	—	—	1.0

^a Sum of the impurities found in *Organic Impurities, Procedure 1* and *Organic Impurities, Procedure 2*.

• LIMIT OF RESIDUAL SOLVENTS (467)

Internal standard solution: 2 μ L/100 mL of isobutyl alcohol in dimethylformamide

Standard solution: 5 μ L/100 mL each of acetone, alcohol, chloroform, diisopropyl ether, and methanol in *Internal standard solution*

System suitability solution: 0.05 μ L/100 mL each of acetone, alcohol, chloroform, diisopropyl ether, and methanol in *Internal standard solution* from the *Standard solution*

Sample solution: 40 mg of Norgestimate and 2 mL of *Internal standard solution*; shake well to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm \times 30-m fused-silica capillary column bonded with a 1- μ m layer of phase G16

Temperatures

Injection port: 180°

Detector: 250°

Column: See Table 3.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
65	—	65	2.5
65	35	100	2
100	30	160	2.5

Carrier gas: Helium

Flow rate: 6 mL/min

Injection mode: Split

Split flow: 16 mL/min

Injection volume: 1 μ L

System suitability

Samples: *Internal standard solution*, *Standard solution*, and *System suitability solution*

[NOTE—The retention time of isobutyl alcohol in the *Internal standard solution* is 4–5 min.]

Suitability requirements

There are no interfering peaks due to dimethylformamide.

Relative standard deviation: NMT 3.0%, determined from the peak response ratio of each solvent to the internal standard, *Standard solution*

Signal-to-noise ratio: NLT 2.0 for alcohol, *System suitability solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each solvent in the portion of Norgestimate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/W) \times D \times V \times 100$$

R_U = peak response ratio of the relevant analyte to the internal standard from the *Sample solution*

R_S = peak response ratio of the relevant analyte to the internal standard from the *Standard solution*

C_S = concentration of each solvent in the *Standard solution* (mL/mL)

W = weight of Norgestimate used to prepare the *Sample solution* (mg)

D = density of each solvent (mg/mL)

V = volume of *Internal standard solution* used to prepare the *Sample solution* (mL)

Acceptance criteria

Option 1: NMT 0.5% each of acetone and alcohol is found, NMT 0.05% of diisopropyl ether is found, NMT 0.006% of chloroform is found, and NMT 0.3% of methanol is found.

Option 2: Meets the requirements in *Residual Solvents* (467)

SPECIFIC TESTS

• OPTICAL ROTATION, Specific Rotation (781S)

Sample solution: 10 mg/mL in chloroform

Acceptance criteria: +40° to +46°

• LOSS ON DRYING (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• USP REFERENCE STANDARDS (11)

USP Deacetylnorgestimate RS

Mixture of (*E*)- and (*Z*)-17-deacetylnorgestimate.

$C_{21}H_{29}NO_2$ 327.46

USP Norgestimate RS

USP Norgestimate Related Compound A RS

Levonorgestrel acetate.

$C_{23}H_{30}O_3$ 354.48

Norgestimate and Ethinyl Estradiol Tablets

DEFINITION

Norgestimate and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of norgestimate ($C_{23}H_{31}NO_3$) and ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Mobile phase: Tetrahydrofuran, methanol, and water (5:2:13)

Internal standard solution: 0.05 mg/mL of dibutyl phthalate in methanol

Standard solution: Dissolve appropriate quantities of USP Ethinyl Estradiol RS and USP Norgestimate RS in a volume of *Internal standard solution* equivalent to 80% of the final volume. Add a volume of water equivalent to 20% of the final volume, and mix to obtain a solution having a known concentration of about 7 µg/mL of ethinyl estradiol and a known concentration of norgestimate similar to that expected in the *Sample solution*. Pass through a suitable filter of 0.45-µm pore size.

Sample solution: Add a number of Tablets, equivalent to 0.35 mg of ethinyl estradiol, to a suitable glass container. Add 10 mL of water, and mix with a vortex mixer until the Tablets are completely disintegrated. Add 40 mL of *Internal standard solution*, and mix with a vortex mixer for at least 23 min. Sonicate the sample for at least 5 min, pass an aliquot through a suitable filter of 0.45-µm pore size, and use the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 5-cm; 5-µm packing L1

Flow rate: 2.1 mL/min

Injection size: 25 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol, (Z)-norgestimate, (E)-norgestimate, and dibutyl phthalate are about 0.5, 1.0, 1.2, and 1.5, respectively.]

Suitability requirements

Resolution: NLT 1.5 between (Z)-norgestimate and (E)-norgestimate

Relative standard deviation: NMT 2.0% for the peak response ratio of ethinyl estradiol, (Z)-norgestimate, and (E)-norgestimate to dibutyl phthalate

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ethinyl estradiol (C₂₀H₂₄O₂) in the portion of Tablets taken:

$$\text{Result} = (R_{UE}/R_{SE}) \times (C_{SE}/C_{UE}) \times 100$$

R_{UE} = ratio of the peak responses of ethinyl estradiol to dibutyl phthalate from the *Sample solution*

R_{SE} = ratio of the peak responses of ethinyl estradiol to dibutyl phthalate from the *Standard solution*

C_{SE} = concentration of USP Ethinyl Estradiol RS in the *Standard solution* (mg/mL)

C_{UE} = nominal concentration of ethinyl estradiol in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of norgestimate (C₂₃H₃₁NO₃) in the portion of Tablets taken:

$$\text{Result} = (C_{SN}/C_{UN}) \times [P_A(R_{UA}/R_{SA}) + P_S(R_{US}/R_{SS})] \times 100$$

C_{SN} = concentration of USP Norgestimate RS in the *Standard solution* (mg/mL)

C_{UN} = nominal concentration of norgestimate in the *Sample solution* (mg/mL)

P_A = fraction of (E)-norgestimate in USP Norgestimate RS

R_{UA} = ratio of the peak responses of (E)-norgestimate to dibutyl phthalate from the *Sample solution*

R_{SA} = ratio of the peak responses of (E)-norgestimate to dibutyl phthalate from the *Standard solution*

P_S = fraction of (Z)-norgestimate in the USP Norgestimate RS

R_{US} = ratio of the peak responses of (Z)-norgestimate to dibutyl phthalate from the *Sample solution*

R_{SS} = ratio of the peak responses of (Z)-norgestimate to dibutyl phthalate from the *Standard solution*

Calculate the ratio of the content of (Z)-norgestimate to ethinyl estradiol in the portion of Tablets taken, for use in the test for *Organic Impurities*:

$$C_Z/C_E = [(C_{SN} \times P_S) \times (R_{US}/R_{SS})]/[C_{SE}(R_{UE}/R_{SE})]$$

The terms are as defined above.

Acceptance criteria: 90.0%–110.0% each of ethinyl estradiol and norgestimate

PERFORMANCE TESTS

• **DISINTEGRATION** (701): 15 min

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Mobile phase, Standard solution, and Sample solution: Prepare as directed in the *Assay*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: 254 nm

Column: 4.6-mm × 5-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol, (Z)-norgestimate, and (E)-norgestimate are about 0.5, 1.0, and 1.2, respectively.]

Suitability requirements

Resolution: NLT 1.5 between (Z)-norgestimate and (E)-norgestimate

Relative standard deviation: NMT 2.0% for the (Z)-norgestimate and (E)-norgestimate peaks

Analysis

Sample: *Sample solution*

Calculate the percentage of any impurity having a relative retention time of about 0.2 or 0.4, relative to the (Z)-norgestimate peak, and detected at 254 nm in the portion of Tablets taken:

$$\text{Result} = (r_U/r_Z) \times (C_Z/C_E) \times F \times 100$$

r_U = peak response for each impurity

r_Z = peak response for (Z)-norgestimate

C_Z/C_E = ratio of (Z)-norgestimate to ethinyl estradiol, as defined in the *Assay*

F = relative response factor of these impurities, 1.54

Acceptance criteria: The sum of the impurities having relative retention times of about 0.2 and 0.4 is NMT 4.0%.

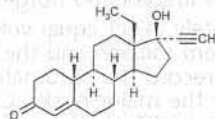
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** (11)

USP Ethinyl Estradiol RS
USP Norgestimate RS

Norgestrel



$C_{21}H_{28}O_2$ 312.45

18,19-Dinorpregn-4-en-20-yn-3-one, 13-ethyl-17-hydroxy-, (17 α)-(±)-.

(±)-13-Ethyl-17-hydroxy-18,19-dinor-17 α -pregn-4-en-20-yn-3-one [6533-00-2].

» Norgestrel contains not less than 98.0 percent and not more than 102.0 percent of $C_{21}H_{28}O_2$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Norgestrel RS

Identification, Infrared Absorption (17K)—If differences appear, dissolve portions of both the test specimen and the Reference Standard in ethyl acetate, evaporate the solutions on a steam bath to dryness, and repeat the test.

Melting range, Class I (741): between 205° and 212°, but the range between beginning and end of melting does not exceed 4°.

Optical rotation (781A): between −0.1° and +0.1°.

Test solution: 50 mg, previously dried, per mL, in chloroform.

Loss on drying (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.3%.

Chromatographic purity—

Phosphomolybdic acid reagent—Add 10 g of phosphomolybdic acid to 100 mL of alcohol, and stir the mixture for not less than 30 minutes. Filter before use.

Test preparation—Prepare a solution of Norgestrel in chloroform to contain 10.0 mg per mL.

Standard solution and Standard dilutions—Prepare a solution of USP Norgestrel RS in chloroform to contain 10 mg per mL (*Standard solution*). Prepare a series of dilutions of *Standard solution* in chloroform to contain 0.20, 0.10, 0.05, 0.02, and 0.01 mg per mL (*Standard dilutions*).

Procedure—Apply 10- μ L volumes of *Standard solution*, the *Test preparation*, and each of the five *Standard dilutions* at equidistant points along a line 2.5 cm from one edge of a 20- \times 20-cm thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture and previously activated by heating at 100° for 15 minutes. Place the plate in a suitable developing chamber that contains and has been equilibrated with a mixture of 96 volumes of chloroform and 4 volumes of alcohol, seal the chamber, and allow the chromatogram to develop until the solvent front has moved 15 cm above the line of application. Remove the plate, allow the solvent to evaporate, then spray uniformly with *Phosphomolybdic acid reagent*, and heat it at 105° for 10 to 15 minutes. The lane of the *Test preparation* exhibits its principal spot at the same R_f as the principal spot of *Standard solution*. If spots other than the principal spot are observed in the lane of the *Test preparation*, estimate the concentra-

tion of each by comparison with the *Standard dilutions*. The spots from the 0.20-, 0.10-, 0.05-, 0.02-, and 0.01-mg per mL dilutions are equivalent to 2.0, 1.0, 0.5, 0.2, and 0.1% of impurities, respectively. The requirements of the test are met if the sum of the impurities in the *Test preparation* does not exceed 2.0%.

Limit of ethynyl group—Proceed as directed in the test for *Limit of ethynyl group* under *Norethindrone*. Not less than 7.81% and not more than 8.18% of ethynyl group is found.

Assay—Dissolve about 100 mg of Norgestrel, accurately weighed, in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a solution containing about 10 μ g per mL. Dissolve an accurately weighed quantity of USP Norgestrel RS in alcohol to obtain a *Standard solution* having a known concentration of about 10 μ g per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 241 nm, with a suitable spectrophotometer, using alcohol as the blank. Calculate the quantity, in mg, of $C_{21}H_{28}O_2$ in the portion of Norgestrel taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in μ g per mL, of USP Norgestrel RS in the *Standard solution*, and A_U and A_S are the absorbances of the solution of Norgestrel and the *Standard solution*, respectively.

Norgestrel Tablets

» Norgestrel Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norgestrel ($C_{21}H_{28}O_2$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Norgestrel RS

Identification—Finely powder 20 Tablets, triturate the powder with 5 mL of chloroform, and allow the solids to settle. Apply 60 μ L of the extract and 60 μ L of a chloroform solution containing about 300 μ g of USP Norgestrel RS per mL at points about 3 cm from one edge of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a developing chamber containing a mixture of chloroform and alcohol (96:4) to a depth of 2 cm, the chamber having been previously equilibrated with the solvent mixture. Remove the plate when the solvent has moved about 15 cm from the line of application, dry at room temperature, spray with a mixture of 80 volumes of sulfuric acid and 20 volumes of alcohol, and heat at 105° for several minutes: the spot from the solution under test exhibits an R_f value identical to that of the spot from the *Standard solution*, and, when viewed under long-wavelength UV light, exhibits a red fluorescence similar to that from the *Standard solution*.

Disintegration (701): 15 minutes, the use of disks being omitted.

Uniformity of dosage units (905): meet the requirements.

Assay—

Isoniazid reagent—Dissolve 0.25 g of isoniazid and 0.3 mL of hydrochloric acid in 500 mL of dehydrated alcohol.

Procedure—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 μ g of norgestrel, to a 30-mL separator containing 5 mL of water. Extract with three 5-mL portions of chloroform, shaking for about 1 minute each

time, and collecting the chloroform extracts through glass wool, previously moistened with chloroform, into a glass-stoppered test tube. Add 1 mL of dilute hydrochloric acid (1 in 12) to the remaining aqueous phase and extract with a fourth 5-mL portion of chloroform, collecting this chloroform extract as before and combining it with the previous three. To another glass-stoppered test tube transfer 20.0 mL of a solution of USP Norgestrel RS, in chloroform, having a known concentration of about 3.75 µg per mL. Evaporate the contents of both tubes in a water bath with the aid of a current of air to dryness. Add 5.0 mL of *Isoniazid reagent* to each tube, insert the stopper in each tube, and swirl occasionally for 1 hour. Concomitantly determine the absorbances of both solutions in 1-cm cells, at the wavelength of maximum absorbance at about 380 nm, using a suitable spectrophotometer, and using *Isoniazid reagent* as the blank. Calculate the quantity, in µg, of $C_{21}H_{28}O_2$ in the portion of Tablets taken by the formula:

$$20C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Norgestrel RS in the Standard solution, and A_U and A_S are the absorbances of the solutions from the Tablets and the Standard solution, respectively.

Norgestrel and Ethinyl Estradiol Tablets

» Norgestrel and Ethinyl Estradiol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norgestrel ($C_{21}H_{28}O_2$) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Ethinyl Estradiol RS

USP Norgestrel RS

Identification—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)—

Medium: 0.0005% (w/v) polysorbate 80; 500 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Determine the amount of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ dissolved by employing the following method. [NOTE—Do not use plastics during the preparation of solutions.]

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard solution [NOTE—A volume of alcohol not exceeding 2% of the final volume of the solution may be used to aid in dissolving the USP Reference Standards.]—Dissolve an accurately weighed quantity of USP Norgestrel RS and USP Ethinyl Estradiol RS in *Medium*, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having known concentrations similar to those expected in the *Test solution*.

Test solution—Use a portion of the solution under test filtered through 0.7-µm borosilicate microfiber filter.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 247-nm detector

(for norgestrel analysis), and a spectrofluorometric detector (for ethinyl estradiol analysis) with an excitation wavelength of about 285 nm and an emission wavelength of 310 nm, and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for ethinyl estradiol and 1.0 for norgestrel; and the relative standard deviation for replicate injections is not more than 3.0% for the ethinyl estradiol and norgestrel peaks.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of norgestrel ($C_{21}H_{28}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved by the formula:

$$(500C)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard solution*; and r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ is dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase—Prepare a degassed mixture of water, acetonitrile, and methanol (45:35:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Norgestrel RS and USP Ethinyl Estradiol RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 100 µg of norgestrel per mL and 10 µg of ethinyl estradiol per mL.

Assay preparation—Transfer an accurately counted number of Tablets, equivalent to about 10 mg of norgestrel, to a 200-mL volumetric flask. Add 100.0 mL of *Mobile phase*, accurately measured, sonicate for 10 minutes to disintegrate the Tablets, and shake by mechanical means for 20 minutes. Centrifuge the clear portion of the solution at about 2000 rpm for 10 minutes, and filter the clear supernatant.

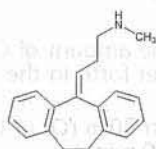
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for ethinyl estradiol and 1.5 for norgestrel; the resolution, R , between the two major peaks is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ethinyl estradiol ($C_{20}H_{24}O_2$) and norgestrel ($C_{21}H_{28}O_2$) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the relevant USP Reference Standard in the *Standard preparation*; and r_U and r_S are the peak responses for the relevant analyte obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nortriptyline Hydrochloride



$C_{19}H_{21}N \cdot HCl$ 299.84
 1-Propanamine, 3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N-methyl-, hydrochloride;
 10,11-Dihydro-N-methyl-5H-dibenzo[a,d]cycloheptene- Δ^5 - γ -propylamine hydrochloride [894-71-3].

DEFINITION

Nortriptyline Hydrochloride contains NLT 97.0% and NMT 101.5% of nortriptyline hydrochloride ($C_{19}H_{21}N \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- A. INFRARED ABSORPTION (17K)**
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- C. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements when tested as specified for alkaloidal hydrochlorides

ASSAY

PROCEDURE

Solution A: Phosphoric acid and water (1:10)

Buffer: Dissolve 1.4 g of dibasic sodium phosphate in 1 L of water, and adjust with *Solution A* to a pH of 7.7.

Mobile phase: Methanol and *Buffer* (70:30)

Standard solution: 0.2 mg/mL of USP Nortriptyline Hydrochloride RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Nortriptyline Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Column temperature: 45°

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

Run time: 1.3 times the retention time of nortriptyline

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of nortriptyline hydrochloride

($C_{19}H_{21}N \cdot HCl$) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Nortriptyline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Nortriptyline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–101.5% on the dried basis

IMPURITIES

- RESIDUE ON IGNITION (281):** NMT 0.1%

Delete the following:

- HEAVY METALS, Method II (231):** NMT 10 ppm (Official 1-

Jan-2018)

ORGANIC IMPURITIES

Solution A: 400 mg/mL of tetrabutylammonium hydroxide in water

Solution B: Phosphoric acid and water (1:7)

Buffer: Dissolve 0.7 g of potassium dihydrogen phosphate in 900 mL water. Add 3.25 mL of *Solution A*, and adjust with *Solution B* to a pH of 7.5. Dilute with water to 1 L.

Mobile phase: Methanol and *Buffer* (75:25). [NOTE—The *Mobile phase* ratio can be adjusted to 70:30 to meet the system suitability requirements.]

System suitability solution: 2.0 μ g/mL each of USP Amitriptyline Related Compound B RS, USP

Cyclobenzaprine Related Compound B RS, and USP

Nortriptyline Hydrochloride RS in *Mobile phase*

Standard solution: 2.0 μ g/mL of USP Nortriptyline Hydrochloride RS in *Mobile phase*

Sample solution: 2.0 mg/mL of Nortriptyline Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Column temperature: 35°. [NOTE—The *Column temperature* can be adjusted to 45° to meet the system suitability requirements.]

Flow rate: 1 mL/min

Injection volume: 10 μ L

Run time: 3 times the retention time of nortriptyline

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.4 between the amitriptyline related compound B peaks; NLT 2.0 between the cyclobenzaprine related compound B and nortriptyline peaks, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Nortriptyline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of nortriptyline from the *Standard solution*

C_S = concentration of USP Nortriptyline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Nortriptyline Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.05% of the area of the principal peak from the *Sample solution*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amitriptyline related compound A ^a	0.5	1.0	0.05
Amitriptyline related compound B	0.8	0.58	0.15
Cyclobenzaprine related compound B	0.9	1.0	0.10
Nortriptyline	1.0	—	—
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.2

^a Dibenzosuberone.**SPECIFIC TESTS**• **LOSS ON DRYING** (731)

Analysis: Dry a sample at 105° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** (11)

USP Amitriptyline Related Compound B RS

5-[3-(Dimethylamino)propyl]-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5-ol.

C₂₀H₂₅NO 295.42

USP Cyclobenzaprine Related Compound B RS

3-(5H-Dibenzo[a,d]cyclohepten-5-ylidene)-N-methyl-1-propanamine hydrochloride.

C₁₉H₁₉N · HCl 297.81

USP Nortriptyline Hydrochloride RS

Nortriptyline Hydrochloride Capsules

» Nortriptyline Hydrochloride Capsules contain nortriptyline hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nortriptyline (C₁₉H₂₁N).

Packaging and storage—Preserve in tight containers.**USP Reference standards** (11)—

USP Nortriptyline Hydrochloride RS

Identification—

A: Transfer the contents of Capsules, equivalent to about 50 mg of nortriptyline hydrochloride, to a suitable flask. Add 15 mL of chloroform, insert the stopper in the flask, and shake for 15 minutes. Transfer the mixture to a suitable centrifuge tube, and centrifuge at about 2900 rpm for about 5 minutes. Pass through a suitable filter paper containing a small amount of anhydrous sodium sulfate. Evaporate the filtrate to dryness, and dissolve the residue in 0.5 mL of chloroform: the IR absorption spectrum of this solution exhibits maxima only at the same wavelengths as that of a Standard solution prepared by dissolving 50 mg of USP Nortriptyline Hydrochloride RS in 0.5 mL of chloroform.

B: A filtered solution in water of the contents of Capsules, equivalent to nortriptyline hydrochloride solution (1 in 20), responds to the tests for *Chloride* (191), when tested as specified for alkaloidal hydrochlorides.

Dissolution (711)—

Medium: water; 500 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of C₁₉H₂₁N dissolved, employing the procedure set forth in the *Assay*, making any necessary modifications.

Tolerances—Not less than 80% (Q) of the labeled amount of C₁₉H₂₁N is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Phosphate buffer—Dissolve 1.63 g of monobasic potassium phosphate in 1 L of water, and adjust with 1 N potassium hydroxide to a pH of 6.7.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, methanol, and *Phosphate buffer* (40:43:17). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Nortriptyline Hydrochloride RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.38 mg per mL.

Assay preparation—Weigh, empty, and combine the contents of not less than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 76 mg of nortriptyline hydrochloride, to a 200-mL volumetric flask, and dissolve in about 150 mL of methanol. Shake by mechanical means for 15 minutes, dilute with methanol to volume, mix, and filter.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 239-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 2.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency is not less than 500 theoretical plates, the tailing factor is not more than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of nortriptyline (C₁₉H₂₁N) in the portion of Capsules taken by the formula:

$$(263.38 / 299.84)(200C)(r_U / r_S)$$

in which 263.38 and 299.84 are the molecular weights of nortriptyline and nortriptyline hydrochloride, respectively; C is the concentration, in mg per mL, of USP Nortriptyline Hydrochloride RS in the *Standard preparation*; and *r_U* and *r_S* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Nortriptyline Hydrochloride Oral Solution

» Nortriptyline Hydrochloride Oral Solution contains nortriptyline hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nortriptyline (C₁₉H₂₁N).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Nortriptyline Hydrochloride RS

Identification—

A: Transfer a measured volume of Oral Solution, equivalent to about 50 mg of nortriptyline hydrochloride, to a suitable separator, and render the solution distinctly alkaline (to a pH of 11 or above as indicated by pH indicator paper) by the dropwise addition of 1 N sodium hydroxide. Extract with 15 mL of chloroform, and filter the chloroform extract through about 2 g of anhydrous sodium sulfate that has been previously washed with chloroform. Evaporate the chloroform extract with the aid of heat and a current of air to dryness, and dissolve the residue in 0.5 mL of chloroform: the IR absorption spectrum of this solution exhibits maxima only at the same wavelengths as that of a Standard solution obtained by dissolving 50 mg of USP Nortriptyline Hydrochloride RS in 25 mL of water and proceeding as directed for the test specimen.

B: It responds to the tests for *Chloride* (191), when tested as specified for alkaloidal hydrochlorides.

Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

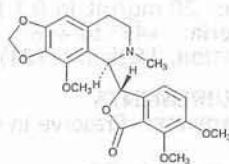
pH (791): between 2.5 and 4.0.

Alcohol Determination, Method II (611): between 3.0% and 5.0% of C_2H_5OH .

Assay—Transfer an accurately measured volume of Oral Solution, equivalent to about 10 mg of nortriptyline, to a 125-mL separator. Add 20 mL of water, mix, and render the solution distinctly alkaline (to a pH of 11 or above as indicated by pH indicator paper) by the dropwise addition of sodium hydroxide solution (1 in 2). Extract the nortriptyline with four 25-mL portions of chloroform, filtering each extract into a 250-mL beaker through about 12 g of anhydrous sodium sulfate previously washed with 25 mL of chloroform. Rinse the sodium sulfate with four 5-mL portions of chloroform, and collect the rinsings in the beaker. Evaporate the combined chloroform solution with the aid of heat and a current of air to about 10 mL. Transfer the contents of the beaker with the aid of chloroform to a 200-mL volumetric flask. Evaporate the chloroform with the aid of air alone to dryness. [Caution—Do not use heat.] Dissolve the residue in 1.7 mL of hydrochloric acid, dilute with water to volume, and mix. Transfer 10.0 mL of the solution to a 50-mL volumetric flask, dilute with water to volume, and mix to obtain the *Assay preparation*. Concomitantly determine the absorbances of the *Assay preparation* and a Standard solution of USP Nortriptyline Hydrochloride RS in water having a known concentration of about 11.4 μ g per mL in 1-cm cells at the wavelength of maximum absorbance at about 239 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{19}H_{21}N$ in the portion of Oral Solution taken by the formula:

$$(263.38/299.84)(C)(A_U / A_S)$$

in which 263.38 and 299.84 are the molecular weights of nortriptyline and nortriptyline hydrochloride, respectively; C is the concentration, in μ g per mL, of USP Nortriptyline Hydrochloride RS in the Standard solution; and A_U and A_S are the absorbances of the *Assay preparation* and the Standard solution, respectively.

Noscapine

$C_{22}H_{23}NO_7$ 413.42

1(3*H*)-Isobenzofuranone, 6,7-dimethoxy-3-(5,6,7,8-tetrahydro-4-methoxy-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl), [*S*-(*R**,*S**)]-;

Narcotine [128-62-1].

DEFINITION

Noscapine contains NLT 99.0% and NMT 100.5% of noscapine ($C_{22}H_{23}NO_7$), calculated on the anhydrous basis.

IDENTIFICATION

- A. INFRARED ABSORPTION (197K):** Do not dry specimens.
- B. ULTRAVIOLET ABSORPTION (197U)**

Solution: 60 μ g/mL

Medium: Methanol

Acceptance criteria: Meets the requirements

C.

Analysis: Place about 100 mg in a small porcelain dish, add a few drops of sulfuric acid, and stir.

Acceptance criteria: A greenish yellow solution is produced, and on warming it becomes red and then turns violet.

ASSAY**PROCEDURE**

Sample solution: Dissolve about 1.5 g of Noscapine in 25 mL of glacial acetic acid.

Analysis: Add 25 mL of dioxane and 5 drops of crystal violet TS to the *Sample solution*, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 41.34 mg of noscapine ($C_{22}H_{23}NO_7$).

Acceptance criteria: 99.0%–100.5% on the anhydrous basis

IMPURITIES

- RESIDUE ON IGNITION (281):** NMT 0.1%
- CHLORIDE AND SULFATE, Chloride (221):** A 700-mg portion shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.02%).

LIMIT OF MORPHINE

Sample solution: Dissolve 100 mg in 10 mL of 0.1 N hydrochloric acid.

Analysis: To 1.0 mL of the *Sample solution* add 5.0 mL of diluted ferricyanide reagent (prepared by dissolving 0.50 g of potassium ferricyanide in 50 mL of water, adding 0.50 mL of ferric chloride TS, and diluting 5.0 mL of the resulting solution to 25.0 mL).

Acceptance criteria: No blue or dark green color develops within 1 min.

ORDINARY IMPURITIES (466)

Standard solution: Chloroform

Test solution: Chloroform

Eluant: Ethyl acetate and ether (80:20)

Visualization: 17; then examine the plate immediately.

Acceptance criteria: The sum of the intensities of all secondary spots of the *Test solution* corresponds to NMT 1.0%.

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** (741): 174°–176°
- **OPTICAL ROTATION**, *Specific Rotation* (781S)
Sample solution: 20 mg/mL in 0.1 N hydrochloric acid
Acceptance criteria: +42° to +48°
- **WATER DETERMINATION**, *Method I* (921): NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)
USP Noscaphine RS

Novobiocin SodiumC₃₁H₃₅N₂NaO₁₁ 634.61

Benzamide, N-[7-[[3-O-(aminocarbonyl)-6-deoxy-5-C-methyl-4-O-methyl-β-L-lyxo-hexopyranosyl]oxy]-4-hydroxy-8-methyl-2-oxo-2H-1-benzopyran-3-yl]-4-hydroxy-3-(3-methyl-2-butenyl), monosodium salt.
Novobiocin, monosodium salt [1476-53-5].

» Novobiocin Sodium has a potency equivalent to not less than 850 μg of novobiocin (C₃₁H₃₆N₂O₁₁) per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Novobiocin RS

Identification—

A: Prepare a test solution by dissolving a quantity of it in methanol to obtain a concentration of about 1 mg of novobiocin per mL. Similarly prepare a Standard solution, using USP Novobiocin RS. Separately apply 1-μL portions of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, and allow the spots to dry. Place the plate in a chromatographic chamber equilibrated with a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (75:25:1), and develop the chromatogram. When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, and allow to dry. Locate the spots on the plate by examination under short-wavelength UV light: the *R_f* value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

B: The residue obtained by igniting it responds to the tests for Sodium (191).

Specific rotation (781S): between –50° and –58°.

Test solution: 50 mg per mL, in a mixture of methanol and hydrochloric acid (100:1).

Crystallinity (695): meets the requirements.

pH (791): between 6.5 and 8.5, in a solution containing 25 mg per mL.

Loss on drying (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 6.0% of its weight.

Residue on ignition (281): between 10.5% and 12.0%, the charred residue being moistened with 2 mL of sulfuric acid and an ignition temperature of 550 ± 50° being used.

Change to read:

Assay—Dissolve a suitable quantity of Novobiocin Sodium, accurately weighed, in an accurately measured volume of

• **Buffer B.3** (CN 1-May-2017) sufficient to obtain a stock solution of convenient concentration. Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock solution diluted quantitatively and stepwise with • **Buffer B.6** (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Novobiocin Sodium Intramammary Infusion

» Novobiocin Sodium Intramammary Infusion is a suspension of Novobiocin Sodium in a suitable vegetable oil vehicle. It contains suitable preservative and suspending agents. It contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of novobiocin (C₃₁H₃₆N₂O₁₁).

Packaging and storage—Preserve in disposable syringes that are well-closed containers.

Labeling—Label it to indicate that it is for veterinary use only.

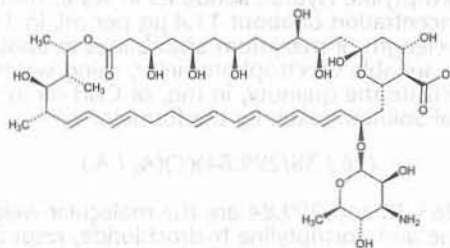
USP Reference standards (11)—

USP Novobiocin RS

Water Determination, *Method I* (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Change to read:

Assay—Proceed as directed for novobiocin under *Antibiotics—Microbial Assays* (81), expelling the contents of a syringe of Intramammary Infusion into a high-speed blender jar containing 1.0 mL of polysorbate 80 and 499.0 mL of • **Buffer B.3** (CN 1-May-2017), and blend for 3 to 5 minutes. Allow to stand for 10 minutes, and dilute quantitatively and stepwise with • **Buffer B.6** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of novobiocin assumed to be equal to the median dose level of the Standard.

NystatinC₄₇H₇₅NO₁₇

Nystatin A;

926.09

14,39-Dioxabicyclo[33.3.1]nonatriaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid, 33-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,4,7,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-, (1S,3R,4R,7R,9R,11R,15S,16R,17R,18S,19E,21E,25E,27E,29E,31E,33R,35S,36R,37S)-; (1S,3R,4R,7R,9R,11R,15S,16R,17R,18S,19E,21E,25E,27E,29E,31E,33R,35S,36R,37S)-33-[(3-Amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,4,7,9,11,17,37-octahydroxy-15,16,

18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nona-triaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid [1400-61-9].

DEFINITION

Nystatin is a substance or a mixture of two or more substances produced by the growth of *Streptomyces noursei* Brown et al. (Fam. Streptomycetaceae). It has a potency of NLT 4400 USP Nystatin Units/mg or, where intended for use in the extemporaneous preparation of oral suspensions, NLT 5000 USP Nystatin Units/mg.

IDENTIFICATION

• A. ULTRAVIOLET ABSORPTION (197U)

Sample stock solution: Transfer a suitable quantity of Nystatin to a suitable glass-stoppered volumetric flask, add methanol and glacial acetic acid, using 25% and 5% of the final volumes respectively, mix to dissolve, dilute with methanol to volume, and mix.

Sample solution: 10 µg/mL of Nystatin from the *Sample stock solution* in methanol

Acceptance criteria: $A_{230}/A_{279(sh)}$, 0.90–1.25

ASSAY

• PROCEDURE

(See *Antibiotics—Microbial Assays* (81).)

Analysis: Proceed as directed in the chapter.

Acceptance criteria: NLT 4400 USP Nystatin Units/mg; where intended for use in the extemporaneous preparation of oral suspensions, NLT 5000 USP Nystatin Units/mg

SPECIFIC TESTS

• SUSPENDABILITY (where packaged for use in the extemporaneous preparation of oral suspensions)

Analysis

1. Transfer about 200 mg, accurately weighed, to a 250-mL beaker containing 200.0 mL of water, and disperse by stirring gently with a stirring rod. Allow to stand for 2 min, and observe the suspension.
2. If there is any sediment, assay the undisturbed suspension as directed for Nystatin in *Antibiotics—Microbial Assays* (81), using a suitable aliquot blended in a high-speed blender for 3–5 min with a sufficient volume of dimethylformamide to obtain a solution having a concentration of 400 USP Nystatin Units/mL. Dilute this stock solution quantitatively with *Buffer B*, 6 to obtain a *Test Dilution* having a nystatin concentration assumed to be equal to the median level of the standard.

Acceptance criteria

1. The material is in suspension, and little or no sediment is present on the bottom of the beaker.
2. If there is any sediment, the undisturbed suspension contains NLT 90.0% of the expected number of USP Nystatin Units, based on the potency obtained in the Assay.

• CRYSTALLINITY (695) (where packaged for use in the extemporaneous preparation of oral suspensions):

Meets the requirements

• pH (791)

Sample suspension: 3% aqueous

Acceptance criteria: 6.0–8.0

• LOSS ON DRYING (731)

Sample: 100 mg

Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

Acceptance criteria: NMT 5.0%

• COMPOSITION

Solution A: Acetonitrile and 0.05 M ammonium acetate (29:71)

Solution B: Acetonitrile and 0.05 M ammonium acetate (60:40)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
25	100	0
35	0	100
40	0	100
45	100	0
50	100	0

System suitability solution: Dissolve 20 mg of Nystatin in 25 mL of methanol, and dilute with water to 50 mL. To 10.0 mL of the resulting solution add 2.0 mL of dilute hydrochloric acid, and allow to stand at room temperature for 1 h.

Standard solution: 0.4 mg/mL of USP Nystatin RS in dimethyl sulfoxide. Protect this solution from light, store refrigerated, and use within 24 h.

Sample solution: 0.4 mg/mL of Nystatin in dimethyl sulfoxide. Protect this solution from light, store refrigerated, and use within 24 h.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 304 nm

Column: 4.6-mm × 15-cm; base-deactivated, end-capped 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The nystatin A1 peak elutes at 14 min. Identify this peak using the *Standard solution*.]

Suitability requirements

Resolution: NLT 3.5 between the two major peaks, *System suitability solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each peak:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = area of each individual peak

r_T = total area of all peaks except those eluting in less than 2 min

Acceptance criteria: NLT 85.0% of nystatin A1; NMT 4.0% of any other individual component. Disregard any peaks eluting in less than 2 min.

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in tight, light-resistant containers.

• LABELING:

Where packaged for use in the extemporaneous preparation of oral suspensions, the label so states.

• USP REFERENCE STANDARDS (11)

USP Nystatin RS

Nystatin Cream

DEFINITION

Nystatin Cream contains NLT 90.0% and NMT 130.0% of the labeled amount of USP Nystatin Units.

ASSAY**• PROCEDURE**

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: 400 USP Nystatin Units/mL prepared as follows. Blend a suitable portion of Cream in a high-speed blender for 3–5 min with a suitable volume of dimethylformamide.

Analysis: Proceed as directed in the chapter. Dilute an aliquot of the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a nystatin concentration assumed to be equal to the median level of the standard.

Acceptance criteria: 90.0%–130.0% of the labeled amount of USP Nystatin Units

PERFORMANCE TESTS

- MINIMUM FILL (755):** Meets the requirements

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in collapsible tubes, or in other tight containers, and avoid exposure to excessive heat.
- USP REFERENCE STANDARDS (11)**
USP Nystatin RS

Nystatin Lotion

» Nystatin Lotion contains not less than 90.0 percent and not more than 140.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in tight containers, at controlled room temperature.

USP Reference standards (11)—

USP Nystatin RS

pH (791): between 5.5 and 7.5.

Assay—Proceed with Lotion as directed in the Assay under *Nystatin Cream*.

Nystatin Lozenges

» Nystatin Lozenges contain not less than 90.0 percent and not more than 125.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Nystatin RS

Disintegration (701): 90 minutes, determined as set forth under *Uncoated Tablets*.

pH (791): between 5.0 and 7.5, in a solution prepared by dissolving 1 Lozenge in 100 mL of water at 37° and allowing the solution to cool to room temperature.

Change to read:

Assay—Proceed as directed for Nystatin under *Antibiotics—Microbial Assays* (81), blending not less than 5 Lozenges for 18 to 20 minutes in a high-speed blender jar containing 100.0 mL of water. Add 400.0 mL of dimethylformamide, and blend for an additional 10 minutes. Dilute an accurately measured volume of this solution quantitatively with a mixture of dimethylformamide and water (4:1) to obtain a stock solution containing about 400 USP Nystatin Units per

mL. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.6* (CN 1-May-2017) to obtain a *Test Dilution* having a nystatin concentration assumed to be equal to the median dose level of the Standard. [NOTE—The *Test Dilution* of the specimen and the test dilutions of the Standard contain the same amount of dimethylformamide (about 4%).]

Nystatin Ointment

» Nystatin Ointment contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in well-closed containers, preferably at controlled room temperature.

USP Reference standards (11)—

USP Nystatin RS

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay—Proceed with Ointment as directed in the Assay under *Nystatin Cream*.

Nystatin Topical Powder

» Nystatin Topical Powder is a dry powder composed of Nystatin and Talc. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Nystatin RS

Loss on drying (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

Assay—Proceed with Topical Powder as directed in the Assay under *Nystatin Cream*.

Nystatin Vaginal Suppositories

» Nystatin Vaginal Suppositories contain not less than 90.0 percent and not more than 130.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in tight, light-resistant containers, at controlled room temperature.

USP Reference standards (11)—

USP Nystatin RS

Water Determination, Method I (921): not more than 1.5%.

Assay—Proceed with Vaginal Suppositories as directed in the Assay under *Nystatin Tablets*.

Nystatin Oral Suspension

» Nystatin Oral Suspension contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of USP Nystatin Units. It contains suitable dispersants, flavors, preservatives, and suspending agents.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Nystatin RS

Uniformity of dosage units (905)—

FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Procedure for content uniformity—[NOTE—Use low-actinic glassware.] Transfer the well-shaken contents of 1 container of Oral Suspension to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Dilute an accurately measured volume of this solution quantitatively, and stepwise if necessary, with methanol to obtain a test solution containing about 25 USP Nystatin Units per mL. Similarly, prepare a Standard solution of USP Nystatin RS in methanol having a known concentration of about 25 USP Nystatin Units per mL. Concomitantly determine the absorbances of the test solution and the Standard solution at the wavelength of maximum absorbance at about 304 nm with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in USP Nystatin Units, in the container taken by the formula:

$$(CL/D)(A_U / A_S)$$

in which C is the concentration, in USP Nystatin Units per mL, of the Standard solution; L is the labeled quantity, in USP Nystatin Units, in the container; D is the concentration, in USP Nystatin Units, in the test solution, on the basis of the labeled quantity in the container and the extent of dilution; and A_U and A_S are the absorbances of the test solution and the Standard solution, respectively.

Deliverable volume (698): meets the requirements.

pH (791): between 4.5 and 6.0; or if it contains glycerin, between 5.3 and 7.5.

Change to read:

Assay—Proceed as directed for Nystatin under *Antibiotics—Microbial Assays* (81), blending a suitable accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, for 3 to 5 minutes in a high-speed blender with a sufficient accurately measured volume of dimethylformamide to obtain a solution of convenient concentration. Dilute an accurately measured portion of this solution quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute this stock solution quantitatively with *Buffer B.6* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Nystatin for Oral Suspension

DEFINITION

Nystatin for Oral Suspension is a dry mixture of Nystatin with one or more suitable colors, diluents, suspending agents, flavors, and preservatives. It contains the equivalent of NLT 90.0% and NMT 140.0% of the labeled amount of USP Nystatin Units.

ASSAY

• PROCEDURE

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: 400 USP Nystatin Units/mL prepared as follows. Constitute Nystatin for Oral Suspension as directed in the labeling. Blend a suitable aliquot of the suspension, freshly mixed and free from air bubbles, for 3–5 min in a high-speed blender with a sufficient volume of dimethylformamide to obtain a solution of suitable concentration. Dilute a portion of the resulting solution with dimethylformamide.

Analysis: Proceed as directed in the chapter. Dilute an aliquot of the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a nystatin concentration assumed to be equal to the median level of the standard.

Acceptance criteria: 90.0%–140.0% of the labeled amount of USP Nystatin Units

PERFORMANCE TESTS

• UNIFORMITY OF DOSAGE UNITS (905)

For powder packaged in single-unit containers

Acceptance criteria: Meets the requirements

• DELIVERABLE VOLUME (698)

For powder packaged in multiple-unit containers

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

• pH (791)

Sample suspension: Constitute as directed in the labeling.

Acceptance criteria: 4.9–5.5

• WATER DETERMINATION, Method I (921): NMT 7.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Nystatin RS

Nystatin Tablets

DEFINITION

Nystatin Tablets contain NLT 90.0% and NMT 130.0% of the labeled amount of USP Nystatin Units.

ASSAY

• PROCEDURE

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: 400 USP Nystatin Units/mL prepared as follows. Blend NLT 5 Tablets for 3–5 min in a high-speed blender with a sufficient volume of dimethylformamide to obtain a solution of suitable concentration. Dilute a portion of this solution with dimethylformamide.

Analysis: Proceed as directed in the chapter. Dilute an aliquot of the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a nystatin concentration assumed to be equal to the median level of the standard.

Acceptance criteria: 90.0%–130.0% of the labeled amount of USP Nystatin Units

PERFORMANCE TESTS

• DISINTEGRATION (701)

Time: If plain-coated, 120 min

Acceptance criteria: Meet the requirements

SPECIFIC TESTS

• LOSS ON DRYING (731)

Sample: 100 mg of powdered Tablets

Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

Acceptance criteria: If plain-coated, NMT 5.0%; if film-coated, NMT 8.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label the Tablets to indicate that they are intended for oral use only (as distinguished from Vaginal Tablets).
- **USP REFERENCE STANDARDS (11)**
USP Nystatin RS

Nystatin Vaginal Inserts**DEFINITION**

Nystatin Vaginal Inserts are composed of Nystatin with suitable binders, diluents, and lubricants. Vaginal Inserts contain NLT 90.0% and NMT 140.0% of the labeled amount of USP Nystatin Units.

ASSAY**Change to read:**• **NYSTATIN**

(See *Antibiotics—Microbial Assays* (81).)

Sample stock solution: 400 USP Nystatin Units/mL in dimethylformamide prepared as follows. Blend NLT 5 Vaginal Inserts for 3–5 min in a high-speed blender with a sufficient volume of dimethylformamide to obtain a solution of suitable concentration. Dilute a portion of this solution with dimethylformamide.

Test dilution: Dilute a volume of the *Sample stock solution* with *Buffer B.6* (CN 1-May-2017) to obtain a nystatin concentration assumed to be equal to the median dose level of the standard.

Acceptance criteria: 90.0%–140.0% of the labeled amount of USP Nystatin Units

PERFORMANCE TESTS• **DISINTEGRATION (701)**

Time: 60 min

Analysis: Use the *Procedure for Uncoated Tablets* in the chapter.

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **LOSS ON DRYING (731):** Dry 100 mg of powdered Vaginal Inserts in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 5.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers and, where so specified in the labeling, in a refrigerator.
- **USP REFERENCE STANDARDS (11)**
USP Nystatin RS

Nystatin, Neomycin Sulfate, Gramicidin, and Triamcinolone Acetonide Cream

» Nystatin, Neomycin Sulfate, Gramicidin, and Triamcinolone Acetonide Cream contains not less than 90.0 percent and not more than 140.0 percent of the labeled amounts of nystatin, neomycin, and gramicidin, and not less than 90.0 percent and not more than 110.0 percent of the

labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Gramicidin RS

USP Neomycin Sulfate RS

USP Nystatin RS

USP Triamcinolone Acetonide RS

Identification—Place 2 g of Cream in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.

Minimum fill (755): meets the requirements.

Change to read:

Assay for nystatin—Proceed as directed for nystatin under *Antibiotics—Microbial Assays* (81), blending a suitable, accurately weighed portion of Cream in a high-speed blender for 3 to 5 minutes with a sufficient, accurately measured volume of dimethylformamide to give a convenient concentration. Dilute an accurately measured volume of the solution so obtained quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.6* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.

Assay for neomycin—Proceed with Cream as directed in the *Assay* under *Neomycin Sulfate Cream*.

Assay for gramicidin—Proceed as directed for gramicidin under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Cream dissolved in 50 mL of hexanes in a separator, and extracted with four 20-mL portions of 80% alcohol. Combine the extracts in a suitable volumetric flask, dilute with alcohol to volume, and mix. Dilute an accurately measured volume of the solution so obtained quantitatively and stepwise with alcohol to obtain a *Test Dilution* having a concentration of gramicidin assumed to be equal to the median dose level of the Standard.

Assay for triamcinolone acetonide—Proceed with Cream as directed in the *Assay* under *Triamcinolone Acetonide Cream*.

Nystatin, Neomycin Sulfate, Gramicidin, and Triamcinolone Acetonide Ointment

» Nystatin, Neomycin Sulfate, Gramicidin, and Triamcinolone Acetonide Ointment contains not less than 90.0 percent and not more than 140.0 percent of the labeled amounts of nystatin, neomycin, and gramicidin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Gramicidin RS

USP Neomycin Sulfate RS

USP Nystatin RS
USP Triamcinolone Acetonide RS

Identification—Place 2 g of Ointment in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Change to read:

Assay for nystatin—Proceed as directed for nystatin under *Antibiotics—Microbial Assays* (81), blending a suitable, accurately weighed portion of Ointment in a high-speed blender for 3 to 5 minutes with a sufficient, accurately measured volume of dimethylformamide to give a convenient concentration. Dilute an accurately measured volume of the solution so obtained quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.6* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.

Assay for neomycin—Proceed with Ointment as directed in the *Assay under Neomycin Sulfate Ointment*.

Assay for gramicidin—Proceed as directed for gramicidin under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Ointment dissolved in 50 mL of hexanes in a separator, and extracted with four 20-mL portions of 80% alcohol. Combine the extracts in a suitable volumetric flask, dilute with alcohol to volume, and mix. Dilute an accurately measured volume of the solution so obtained quantitatively and stepwise with alcohol to obtain a *Test Dilution* having a concentration of gramicidin assumed to be equal to the median dose level of the Standard.

Assay for triamcinolone acetonide—Proceed with Ointment as directed in the *Assay under Triamcinolone Acetonide Cream*, except to read "Ointment" in place of "Cream" throughout.

Nystatin, Neomycin Sulfate, Thiostrepton, and Triamcinolone Acetonide Cream

» Nystatin, Neomycin Sulfate, Thiostrepton, and Triamcinolone Acetonide Cream contains not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of nystatin, neomycin, and thiostrepton, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Nystatin RS
USP Neomycin Sulfate RS
USP Thiostrepton RS
USP Triamcinolone Acetonide RS

Identification—Place 2 g of Cream in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.

Minimum fill (755): meets the requirements.

Change to read:

Assay for nystatin—Proceed as directed for nystatin under *Antibiotics—Microbial Assays* (81), blending a suitable, accurately weighed portion of Cream in a high-speed blender for 3 to 5 minutes with a sufficient, accurately measured volume of dimethylformamide to give a convenient concentration. Dilute an accurately measured volume of the solution so obtained quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.6* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for neomycin—Proceed as directed for the turbidimetric assay for neomycin under *Antibiotics—Microbial Assays* (81), placing an accurately weighed portion of Cream, equivalent to about 2.5 mg of neomycin, in a 250-mL conical flask, and treating it as follows. Add 50 mL of 0.01 N hydrochloric acid, and shake to disperse the Cream. Transfer the mixture to a 100-mL centrifuge tube. Wash the flask with 40 mL of hexanes, with shaking, and transfer the washing to the centrifuge tube. Stopper the centrifuge tube, shake, and centrifuge for 5 minutes. Draw off the lower aqueous layer, and transfer it to a 250-mL volumetric flask. Repeat the extraction of the hexanes layer remaining in the centrifuge tube with two 50-mL portions of 0.01 N hydrochloric acid, combining the aqueous extracts in the 250-mL volumetric flask. Dilute the contents of the volumetric flask with 0.01 N hydrochloric acid to volume, and mix. Dilute this solution quantitatively and stepwise with *Buffer B.3* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose of the Standard.

Assay for thiostrepton—Proceed as directed for thiostrepton under *Antibiotics—Microbial Assays* (81), blending a suitable, accurately weighed portion of Cream in a high-speed blender with a sufficient, accurately measured volume of dimethyl sulfoxide to give a convenient concentration, and filter. Dilute an accurately measured volume of the filtrate so obtained quantitatively with dimethyl sulfoxide to obtain a *Test Dilution* having a concentration of thiostrepton assumed to be equal to the median dose level of the Standard.

Assay for triamcinolone acetonide—Proceed with Cream as directed in the *Assay under Triamcinolone Acetonide Cream*.

Nystatin, Neomycin Sulfate, Thiostrepton, and Triamcinolone Acetonide Ointment

» Nystatin, Neomycin Sulfate, Thiostrepton, and Triamcinolone Acetonide Ointment contains not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of nystatin, neomycin, and thiostrepton, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Nystatin RS

USP Neomycin Sulfate RS

USP Thiostrepton RS

USP Triamcinolone Acetonide RS

Identification—Place 2 g of Ointment in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.

Minimum fill (755): meets the requirements.

Assay for nystatin—[NOTE—Protect solutions that contain nystatin from ambient light.]

Ammonium acetate buffer—Dissolve 10.8 ± 1.0 g of ammonium acetate in 2500 mL of water. Adjust with acetic acid to a pH of 6.50 ± 0.05 .

Mobile phase—Mix 2500 mL of *Ammonium acetate buffer*, 1000 mL of acetonitrile, and 500 mL of methanol. Pass through a 0.45-µm nylon filter.

BHT solution—Weigh about 1.0 g of butylated hydroxytoluene, and transfer to a 1000-mL volumetric flask. Dilute with methanol to volume, and mix.

Standard preparation—In duplicate, dissolve an accurately weighed quantity of USP Nystatin RS in *BHT solution* to obtain a solution having a known concentration of about 5400 USP Nystatin Units per mL. Store in low-actinic glassware.

System suitability solution—Weigh about 50 mg of USP Nystatin RS, and transfer to a 50-mL low-actinic volumetric flask. Add 0.5 mL of 0.01 N sodium hydroxide, and allow to sit for 1 minute. Add 5 mL of *Ammonium acetate buffer*. Add about 25 mL of methanol, and sonicate to dissolve. Dilute with methanol to volume, and store in low-actinic glassware.

Assay preparation—Thoroughly mix the Ointment prior to sampling. In duplicate, accurately weigh about 1.0 g of Ointment having a known density into a low-actinic sample bottle. Add 20.0 mL of *BHT solution*, and insert a polytetrafluoro-coated magnetic stir bar having dimensions of about 12.7×7.9 mm. Clamp the bottles onto a suitable mixer mill,¹ and mix for a minimum of 5 minutes at about 30 Hz. Centrifuge at about $1350 \times g$ for 5 minutes, or until the supernatant is clear. Transfer the supernatant to low-actinic glassware.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 304-nm detector

and a 3.9-mm \times 15-cm column that contains 4-µm packing L1. The column temperature is maintained at 40°, and the flow rate is about 2.0 mL per minute. [NOTE—Solutions containing nystatin should be stored at 8° until they can be injected into the chromatograph.] Chromatograph the *Standard preparation* and the *System suitability solution*, and record the peak areas as directed for *Procedure*: using the *System suitability solution*, the relative retention times for the nystatin A1 and nystatin A2 peaks are about 1.0 and 1.4, respectively; the column efficiency, using the nystatin A1 peak, is not less than 1200 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 3.0%. [NOTE—After the conclusion of the run, rinse the column with a mixture of acetonitrile and water (85:15) until the baseline is stable, and store in this solution. At the beginning of the next run, rinse with *Mobile phase* until the baseline is stable.]

Procedure—Separately inject equal volumes (about 15 µL) of the duplicate *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for nystatin A1 and nystatin A2. Calculate the quantity, in USP Nystatin Units, of nystatin in the portion of Ointment taken by the formula:

$$20(C_s / W_u)(r_u / r_s)$$

in which C_s is the concentration of USP Nystatin RS, in USP Nystatin Units per mL, of the *Standard preparation*; W_u is the weight, in g, of Ointment taken to prepare the *Assay preparation*; and r_u and r_s are the average peak areas of the sum of nystatin A1 and nystatin A2 obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Change to read:

Assay for neomycin—Proceed as directed for the turbidimetric assay for neomycin under *Antibiotics—Microbial Assays* (81), placing an accurately weighed portion of Ointment, equivalent to about 2.5 mg of neomycin, in a 250-mL conical flask, and treating it as follows. Add 50 mL of hexanes, and shake to disperse the Ointment. Transfer the mixture to a 250-mL separator. Wash the flask with 50 mL of 0.01 N hydrochloric acid, with shaking, and transfer the washing to a separator. Stopper the separator, shake, and allow the layers to separate. Draw off the lower aqueous layer, collecting it in a 250-mL volumetric flask. Repeat the extraction of the hexanes layer remaining in the separator with two or more 50-mL portions of 0.01 N hydrochloric acid, combining the aqueous extracts in the 250-mL volumetric flask. Dilute the contents of the volumetric flask with 0.01 N hydrochloric acid to volume, and mix. Dilute this solution quantitatively and stepwise with *Buffer B.3* (CN 1, May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose of the Standard.

Assay for thiostrepton—Proceed as directed for thiostrepton under *Antibiotics—Microbial Assays* (81), blending a suitable, accurately weighed portion of Ointment in a high-speed blender with a sufficient, accurately measured volume of dimethyl sulfoxide to give a convenient concentration, and filter. Quantitatively dilute an accurately measured volume of the filtrate so obtained with dimethyl sulfoxide to obtain a *Test Dilution* having a concentration of thiostrepton assumed to be equal to the median dose of the Standard.

Assay for triamcinolone acetonide—Proceed with Ointment as directed in the *Assay* under *Triamcinolone Acetonide Cream*, but read "Ointment" in place of "Cream" throughout.

¹A suitable mixer mill can be obtained from Retsch Inc., 74 Walker Lane, Newtown, PA 18940 (www.retsch-us.com; 267-757-0351), product number MM 301.

Nystatin and Triamcinolone Acetonide Cream

» Nystatin and Triamcinolone Acetonide Cream contains not less than 90.0 percent and not more than 140.0 percent of the labeled amount of USP Nystatin Units and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Nystatin RS

USP Triamcinolone Acetonide RS

Identification—Place 2 g of Cream in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.

Minimum fill (755): meets the requirements.

Change to read:

Assay for nystatin—Proceed as directed for nystatin under *Antibiotics—Microbial Assays* (81), blending a suitable, accurately weighed portion of Cream in a high-speed blender for 3 to 5 minutes with a sufficient, accurately measured volume of dimethylformamide to give a convenient concentration. Dilute an accurately measured volume of the solution so obtained quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.6* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.

Assay for triamcinolone acetonide—Proceed with Cream as directed in the *Assay* under *Triamcinolone Acetonide Cream*.

Nystatin and Triamcinolone Acetonide Ointment

» Nystatin and Triamcinolone Acetonide Ointment contains not less than 90.0 percent and not

more than 140.0 percent of the labeled amount of USP Nystatin Units and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Nystatin RS

USP Triamcinolone Acetonide RS

Identification—Place 2 g of Ointment in a conical flask, add 5.0 mL of chloroform, and shake for 10 minutes. Add 15 mL of alcohol, and shake for an additional 10 minutes. Filter the solution into a centrifuge tube, and evaporate the filtrate to dryness. Dissolve the residue in alcohol to obtain a solution containing about 250 µg of triamcinolone acetonide per mL. Proceed as directed in the *Identification* test under *Triamcinolone Acetonide Cream*, beginning with "Apply 10 µL of this solution": the specified result is observed.

Minimum fill (755): meets the requirements.

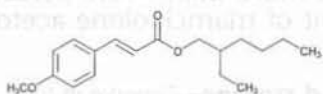
Water Determination, Method I (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Change to read:

Assay for nystatin—Proceed as directed for nystatin under *Antibiotics—Microbial Assays* (81), blending a suitable, accurately weighed portion of Ointment in a high-speed blender for 3 to 5 minutes with a sufficient, accurately measured volume of dimethylformamide to give a convenient concentration. Dilute an accurately measured volume of the solution so obtained quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute an accurately measured volume of this stock solution quantitatively with *Buffer B.6* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.

Assay for triamcinolone acetonide—Proceed with Ointment as directed in the *Assay* under *Triamcinolone Acetonide Cream*, except to read "Ointment" in place of "Cream" throughout.

Octinoxate



$C_{18}H_{26}O_3$ 290.40
2-Ethylhexyl 3-(4-methoxyphenyl)-2-propenoate.
2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester.
[5466-77-3].

» Octinoxate contains not less than 95.0 percent and not more than 105.0 percent of $C_{18}H_{26}O_3$, calculated on the as-is basis.

Packaging and storage—Preserve in tight containers, in a cool place.

USP Reference standards (11)—

USP Octinoxate RS
Octyl methoxycinnamate.

Identification—

A: Infrared Absorption (197F).

B: Ultraviolet Absorption (197U)—

Solution: 5 μ g per mL.

Medium: alcohol.

Specific gravity (841): between 1.005 and 1.013.

Refractive index (831): between 1.542 and 1.548 at 20°.

Acidity—Transfer 5 mL of Octinoxate to a suitable container, add 50 mL of alcohol, and mix. Add 4 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide: not more than 0.8 mL is consumed.

Chromatographic purity—

Test solution—Transfer about 5 mL of Octinoxate to a 100-mL volumetric flask, dilute with acetone to volume, and mix.

Chromatographic system (see Chromatography (621))—Proceed as directed in the Assay.

Procedure—Inject a volume (about 1 μ L) of the Test solution into the chromatograph, record the chromatogram, and measure the areas for all the peaks. Calculate the percentage of each impurity in the portion of Octinoxate taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak area for each impurity; and r_s is the sum of the areas for all the peaks: not more than 0.5% of any individual impurity is found; and not more than 2.0% of total impurities is found.

Assay—

Internal standard solution—Transfer about 25 mL of benzyl benzoate to a 500-mL volumetric flask, dilute with acetone to volume, and mix.

Standard preparation—Dilute an accurately measured quantity of USP Octinoxate RS quantitatively, and stepwise if necessary, with Internal standard solution to obtain a solution having a known concentration of about 50 mg per mL.

Assay preparation—Transfer about 5 mL of Octinoxate, accurately measured, to a 100-mL volumetric flask, dilute with Internal standard solution to volume, and mix.

Chromatographic system (see Chromatography (621))—The gas chromatograph is equipped with a flame-ionization detector, a 0.32-mm \times 25-m column with 0.25- μ m thickness of phase G1 coating, and a split injection system with a split ratio of about 85:1. The carrier gas is helium, flowing at a rate of about 2 mL per minute. The chromatograph is programmed as follows. Initially the temperature of the column is equilibrated at 80°, then the temperature is increased to

300° over a period of 10 minutes, and maintained at 300° for 10 minutes. The injection port temperature is maintained at 250°, and the detector temperature is maintained at 300°. Chromatograph the Standard preparation, and record the peak areas as directed for Procedure: the relative retention times are about 0.68 for benzyl benzoate and 1.0 for octinoxate; the resolution, R , between benzyl benzoate and octinoxate is not less than 20; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 1 μ L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{26}O_3$ in the portion of Octinoxate taken by the formula:

$$100C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Octinoxate RS in the Standard preparation; and R_U and R_S are the peak area ratios of octinoxate to benzyl benzoate obtained from the Assay preparation and the Standard preparation, respectively.

Octisalate

$C_{15}H_{22}O_3$ 250.33
2-Ethylhexyl salicylate.
Benzoic acid, 2-hydroxy-, 2-ethylhexyl ester [118-60-5].

» Octisalate contains not less than 95.0 percent and not more than 105.0 percent of $C_{15}H_{22}O_3$.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Octisalate RS
Octyl salicylate.

Identification—

A: Infrared Absorption (197F).

B: Ultraviolet Absorption (197U)—

Solution: 5.0 μ g per mL.

Medium: alcohol.

Absorptivity at 305 nm, calculated on the as-is basis, does not differ by more than 3.0%.

Specific gravity (841): between 1.011 and 1.016.

Refractive index (831): between 1.500 and 1.503 at 20°.

Acidity—Transfer 50 mL of alcohol to a suitable container, add 1 mL of phenol red TS, and add sufficient 0.1 N sodium hydroxide to obtain a persistent pink color. Transfer 50 mL of this solution to a suitable container, add about 5.0 mL of accurately measured Octisalate, mix, and titrate with 0.1 N sodium hydroxide: not more than 0.2 mL of 0.1 N sodium hydroxide per mL of Octisalate is required for neutralization.

Chromatographic purity—

Test solution—Use the Assay preparation.

Chromatographic system—Proceed as directed in the Assay. To evaluate the system suitability requirements, use the Standard preparation, as prepared in the Assay.

Procedure—Inject a volume (about 1 μ L) of the Test solution into the chromatograph, record the chromatogram, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Octisalate taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response for each impurity, and r_s is the sum of the responses of all the peaks: not more than

0.5% of any individual impurity is found; and not more than 2.0% of total impurities is found.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Octisalate RS in *tert*-butyl methyl ether, and dilute quantitatively, and stepwise if necessary, with *tert*-butyl methyl ether to obtain a solution having a known concentration of about 20.0 mg per mL.

Assay preparation—Transfer about 2 g of Octisalate, accurately weighed, to a 100-mL volumetric flask, dilute with *tert*-butyl methyl ether to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm × 25-m column coated with a 0.1-μm film of phase G1. The carrier gas is helium, flowing at a rate of about 6 mL per minute. The split ratio is 50:1. [NOTE—Split ratio can be modified in order to optimize the performance.] The chromatograph is programmed as follows. Initially the temperature of the column is equilibrated at 60°, then the temperature is increased at a rate of 8° per minute to 240°, and is maintained at 240° for 10 minutes. The injection port temperature is maintained at 240°, and the detector temperature is maintained at 260°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R , between octisalate and any other peak is not less than 1.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 1 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{15}H_{22}O_3$ in the portion of Octisalate taken by the formula:

$$100C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Octisalate RS in the *Standard preparation*; and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Octocrylene

$C_{24}H_{27}NO_2$ 361.48

2-Propenoic acid, 2-cyano-3,3-diphenyl, 2-ethylhexyl ester.
2-Ethylhexyl 2-cyano-3,3-diphenylacrylate [6197-30-4].

» Octocrylene contains not less than 95.0 percent and not more than 105.0 percent of $C_{24}H_{27}NO_2$.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Octocrylene RS

Identification, Ultraviolet Absorption (197U)—

Solution: 25 μg per mL.

Medium: methanol.

Absorptivity at 303 nm, calculated on the as-is basis, do not differ by more than 3.0%.

Specific gravity (841): between 1.045 and 1.055.

Refractive index (831): between 1.561 and 1.571 at 20°.

Acidity—Transfer 60 mL of alcohol to a suitable container, add 1 mL of phenolphthalein TS, and add sufficient 0.1 N

sodium hydroxide to obtain a persistent pink color. Transfer 60 mL of this solution to a suitable container, add about 6 g of Octocrylene, accurately weighed, mix, and titrate with 0.1 N sodium hydroxide: not more than 0.18 mL of titrant per g of Octocrylene is necessary to obtain a persistent pink endpoint.

Chromatographic purity—

Test solution—Use the *Assay preparation*.

Chromatographic system—Proceed as directed in the *Assay*. To evaluate the system suitability requirements, use the *Standard preparation* prepared as directed in the *Assay*.

Procedure—Inject a volume (about 1 μL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Octocrylene taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks: not more than 0.5% of any individual impurity is found; and not more than 2.0% of total impurities is found.

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Octocrylene RS in acetone, and dilute quantitatively, and stepwise if necessary, with acetone to obtain a solution having a known concentration of about 21.0 mg per mL.

Assay preparation—Transfer about 2.1 g of Octocrylene, accurately weighed, to a 100-mL volumetric flask, dilute with acetone to volume, and mix.

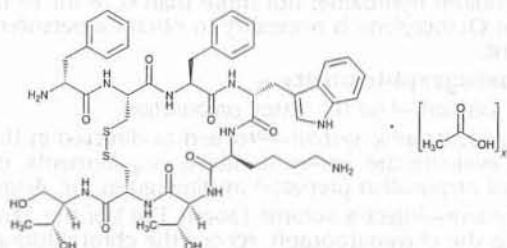
Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm × 60-m column coated with a 0.25-μm film of G1. Helium is used as the carrier gas at a flow rate of about 6 mL per minute. The split ratio is 30:1. The chromatograph is programmed as follows. Initially the temperature of the column is equilibrated at 80°; upon injection, the temperature is increased at a rate of 4° per minute to 280°, and is held at 280° for 10 minutes. The injection port temperature is maintained at 300°, and the detector temperature is maintained at 300°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R , between the octocrylene and any other peak is not less than 1.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 1 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{24}H_{27}NO_2$ in the portion of Octocrylene taken by the formula:

$$100C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Octocrylene RS in the *Standard preparation*; and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Octreotide Acetate



$C_{49}H_{66}N_{10}O_{10}S_2 \cdot xC_2H_4O_2$ 1019 (as free base)
 L-Cysteinamide, D-phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-N-[2-hydroxy-1-(hydroxymethyl)propyl]-, cyclic (2→7)-disulfide, [R-(R*, R*)]-, acetate (salt);
 D-Phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-N-[(1R,2R)-2-hydroxy-1-(hydroxymethyl)propyl]-L-cysteinamide cyclic (2→7)-disulfide acetate (salt);
 D-Phenylalanyl-L-hemicystyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-L-hemicystyl-L-threoninol cyclic (2→7)-disulfide acetate (salt) [79517-01-4].

DEFINITION

Octreotide Acetate is a long-acting synthetic octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. It contains NLT 95.0% and NMT 105.0% of octreotide ($C_{49}H_{66}N_{10}O_{10}S_2$), calculated on the anhydrous, acetic acid-free basis.

IDENTIFICATION

• A. HPLC

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Identification sample solution: Mix an equal volume of the *Standard solution* and the *Sample solution*.

Analysis

Samples: *Identification sample solution, Standard solution, and Sample solution*

Examine the chromatograms of the *Identification sample solution, Standard solution, and Sample solution*.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, and the major peak of the *Identification sample solution* elutes as a single peak.

• B. AMINO ACID ANALYSIS

[NOTE—The following method is given for informational purposes; any validated amino acid analysis method can be used.]

Diluent: 0.1 M hydrochloric acid

Standard amino acid mixture: 2500 nmol/mL of Lys, His, NH₃, Arg, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, and 1250 nmol/mL of Cys-Cys in Diluent

Standard tryptophan solution: 0.2042 mg/mL of tryptophan in Diluent. This solution contains 1000 nmol/mL of Trp.

Standard threoninol solution: 0.1052 mg/mL of Thr-ol in Diluent. This solution contains 1000 nmol/mL Thr-ol. [NOTE—1000 nmol/mL = 1 mM]

Standard solution: Diluent, *Standard amino acid mixture, Standard tryptophan solution, and Standard threoninol solution* (76:4:10:10). The concentration is 100.0 nmol/mL for each amino acid.

Sample solution A: Weigh 1.00–1.50 mg of Octreotide Acetate into a 5-mL ampule. Add 1000 µL of Diluent and 1.2 mL of 30% (w/w) hydrochloric acid, cool the ampule in dry ice, evacuate, and seal by melting. Heat the ampule for 16 h at 115°. Allow to cool and transfer

the contents of the ampule quantitatively into a 25-mL round-bottomed flask, rinse with Diluent, and evaporate to dryness on a rotary evaporator. Dissolve the residue in 10.00 mL of Diluent.

Sample solution B: Weigh 1.00–1.50 mg of Octreotide Acetate into a 5-mL ampule. Add 1000 µL of Diluent and 1.2 mL of 30% (w/w) hydrochloric acid with 1% (v/v) thioglycolic acid, cool the ampule in dry ice, evacuate, and seal by melting. Heat the ampule for 16 h in a heating block at 115°. Allow to cool and transfer the contents of the ampule quantitatively into a 25-mL round-bottomed flask, rinse with Diluent, and evaporate to dryness on a rotary evaporator. Dissolve the residue in 10.00 mL of Diluent.

Analysis

Samples: *Standard solution, Sample solution A, and Sample solution B*

Standardize the instrument with the *Standard solution*.

Inject a suitable volume of *Standard solution, Sample solution A, and Sample solution B*. Evaluate the peak areas of each amino acid found in relation to the peak areas of the respective amino acids in the *Standard solution*, express the content of each amino acid in nmoles.

Calculate A, the average number of nmoles of the amino acids found to be stable under hydrolysis conditions (three stable amino acids—2 Phe and 1 Lys) taken:

$$A = N_T/3$$

N_T = total nmoles of the stable amino acids

Calculate the ratio of the amino acids taken:

$$\text{Result} = N_E/A$$

N_E = nmoles of each amino acid

[NOTE—For Trp use only data obtained with *Sample solution B*. For Cys use only data obtained with *Sample solution A*.]

Acceptance criteria: See Table 1.

Table 1

Name	Acceptance Criteria
Thr	0.7–1.1
Lys	0.9–1.3
Phe	1.8–2.2
Trp	0.4–1.1
Cys	1.0–2.2
Thr-ol	0.6–1.3

ASSAY

• PROCEDURE

Solution A: 0.02% (v/v) of trifluoroacetic acid in water

Solution B: Acetonitrile

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
25	65	35
30	10	90
35	10	90
40	90	10
45	90	10

System suitability solution: 0.5 mg/mL of USP Octreotide Acetate RS and 0.2 mg/mL of USP Octreotide Non-Cyclic System Suitability Marker RS in *Solution A*

Standard solution: 0.5 mg/mL of USP Octreotide Acetate RS in *Solution A*

Sample solution: 0.5 mg/mL of Octreotide Acetate in *Solution A*. [NOTE—Place Octreotide Acetate in a desiccator containing saturated sodium chloride solution for at least 30 min before weighing. Determine the water content by suitable analysis.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 4-μm packing L87

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The retention times for octreotide and non-cyclic octreotide in the *System suitability solution* are about 16.5 and 18.5 min, respectively.]

Suitability requirements

Resolution: NLT 2.0 between octreotide and non-cyclic octreotide, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of octreotide (C₄₉H₆₆N₁₀O₁₀S₂) in the portion of Octreotide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times [100/(100 - W - Ac)] \times 100$$

r_U = peak response of octreotide from the *Sample solution*

r_S = peak response of octreotide from the *Standard solution*

C_S = concentration of USP Octreotide Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Octreotide Acetate in the *Sample solution* (mg/mL)

W = water content in the Octreotide Acetate sample (%)

Ac = acetic acid content in the Octreotide Acetate sample (%)

Acceptance criteria: 95.0%–105.0% on the anhydrous, acetic acid-free basis

OTHER COMPONENTS

- **ACETIC ACID IN PEPTIDES** (503): 5.0%–12.8%

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• OCTREOTIDE ACETATE RELATED COMPOUNDS

[NOTE—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity taken, disregarding any peak with a retention time of less than 5 min and any peak with an area less than 0.1% of the main peak:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the peak responses from the *Sample solution*, excluding those of the solvent peaks

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acetyl-Lys ⁵ -octreotide ^a	1.4	0.5
Acetyl-Phe ¹ -octreotide ^b	1.5	0.5
Unspecified impurity	—	0.5
Total impurities	—	2.0

^a D-Phenylalanyl-L-hemicystyl-L-phenylalanyl-D-tryptophyl-(N-acetyl)-L-lysyl-L-threonyl-L-hemicystyl-L-threonyl cyclic (2→7)-disulfide.

^b (N-Acetyl)-D-Phenylalanyl-L-hemicystyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-L-hemicystyl-L-threonyl cyclic (2→7)-disulfide.

PROCESS-RELATED IMPURITIES

- **TRIFLUOROACETIC ACID (TFA) IN PEPTIDES** (503.1): NMT 0.25%.

[NOTE—Perform this test if trifluoroacetic acid is used in the manufacturing process.]

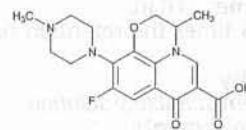
SPECIFIC TESTS

- **WATER DETERMINATION** (921), *Method I*: NMT 10.0%
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 466 USP Endotoxin Units/mg of octreotide acetate
- **MICROBIAL ENUMERATION TESTS** (61) AND **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count is NMT 100 cfu/g. The total yeast and mold count is NMT 100 cfu/g.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in air-tight containers. Store at a temperature of 2°–8°, protected from light.
- **USP REFERENCE STANDARDS** (11)
 - USP Endotoxin RS
 - USP Octreotide Acetate RS
 - USP Octreotide Non-Cyclic System Suitability Marker RS

Ofloxacin



C₁₈H₂₀FN₃O₄ 361.37
 7H-Pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-, (±);
 (±)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid [82419-36-1].

DEFINITION

Ofloxacin contains NLT 98.5% and NMT 101.5% of ofloxacin (C₁₈H₂₀FN₃O₄), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B. ULTRAVIOLET ABSORPTION (197U)**
Medium: 0.1 N perchloric acid
Sample solution: 6.7 µg/mL in Medium
Acceptance criteria: Meets the requirements

ASSAY• **PROCEDURE**

Sample: 100 mg of Ofloxacin
Analysis: Dissolve the *Sample* in 275 mL of acetic anhydride. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass-silver chloride electrode system (see *Titrimetry* (541)). Use the first of the two inflection points. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 36.138 mg of $C_{18}H_{20}FN_3O_4$.
Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%
- **ARSENIC, Method II (211):** NMT 1 µg/g

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 10 µg/g (Official 1, Jan-2018)

• **ORGANIC IMPURITIES**

Diluent: Acetonitrile and water (1:6)
Buffer: 3.1 g/L of ammonium acetate and 5.4 g/L of sodium perchlorate in water. Adjust with phosphoric acid to a pH of 2.2.
Mobile phase: Acetonitrile and *Buffer* (240:1300)
System suitability solution: 0.4 µg/mL each of USP Ofloxacin RS and USP Ofloxacin Related Compound A RS in *Diluent*
Standard solution: 0.4 µg/mL of USP Ofloxacin RS in *Diluent*

Sample solution: 0.2 mg/mL of Ofloxacin in *Diluent*
Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 294 nm

Column: 4.6-mm × 15-cm; packing L1

Column temperature: 45°

Flow rate: 0.5 mL/min

Injection volume: 10 µL

Run time: 2.5 times the retention time of the ofloxacin peak

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between ofloxacin and ofloxacin related compound A

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response of ofloxacin from the *Standard solution*

C_S = concentration of USP Ofloxacin RS in the *Standard solution* (µg/mL)

C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria

Individual impurities: NMT 0.3%. Disregard any peak less than 0.02%.

Total impurities: NMT 0.5%

• **LIMIT OF METHANOL AND ETHANOL**

Internal standard solution: 5.6 mL/mL of *n*-propyl alcohol in sodium hydroxide solution (1 in 100)

Standard solution: 10.0 µg/mL each of methanol and dehydrated alcohol in *Internal standard solution*. Transfer 2 mL of this solution to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 90° for 2 min, and shake for 6 min.

Sample solution: Transfer 40 mg of Ofloxacin to a vial fitted with a septum and crimp cap, add 2 mL of *Internal standard solution*, and seal the vial. Heat the sealed vial at 90° for 2 min, and shake for 6 min.

Blank: Transfer 2 mL of *Internal standard solution* to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 90° for 2 min, and shake for 6 min.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m fused silica column, coated with a 3.0-µm film of stationary phase G43, and a fused silica precolumn

Temperatures

Injector: 170°

Detector: 250°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
35	—	35	3
35	20	90	—
90	40	200	2

Condition the column with the helium flowing at 200° for 2 h or until a stable baseline is obtained.

Carrier gas: Helium

Flow rate: 7 mL/min

Injection volume: 1-mL headspace. Use a heated, gas-tight syringe to make injections of the headspace into the chromatograph.

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for methanol, ethanol, and *n*-propyl alcohol are 0.5, 0.6, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between methanol and ethanol

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*
Calculate the percentage of methanol and ethanol in the portion of Ofloxacin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of methanol or ethanol to the internal standard, corrected for the blank, from the *Sample solution*

R_S = peak response ratio of methanol or ethanol to the internal standard, corrected for the blank, from the *Standard solution*

C_S = concentration of methanol or ethanol in the *Standard solution* (µg/mL)

C_U = concentration of Ofloxacin in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.005% of methanol and NMT 0.05% of ethanol

SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation (781S)**
Sample solution: 10 mg/mL in chloroform
Acceptance criteria: $+1^\circ$ to -1°
- **LOSS ON DRYING (731)**
Analysis: Dry at 105° for 4 h.
Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at 25° , excursions permitted between 15° and 30° .
- **USP REFERENCE STANDARDS (11)**
USP Ofloxacin RS
USP Ofloxacin Related Compound A RS
9-Fluoro-3-methyl-7-oxo-10-(piperazin-1-yl)-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid.
 $C_{17}H_{18}FN_3O_4$ 347.34

Ofloxacin Ophthalmic Solution**DEFINITION**

Ofloxacin Ophthalmic Solution is a sterile aqueous solution of Ofloxacin. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ofloxacin ($C_{18}H_{20}FN_3O_4$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**
Standard stock solution: 3.0 mg/mL of USP Ofloxacin RS in a mixture of chloroform and methanol (1:1)
Standard solution: 0.3 mg/mL of USP Ofloxacin RS from Standard stock solution prepared as follows. Transfer 5.0 mL of Standard stock solution to a 50-mL volumetric flask, add 5 mL of water, and dilute with a mixture of chloroform and methanol (1:1) to volume.
Sample solution: 0.3 mg/mL of ofloxacin from a portion of Ophthalmic Solution in a mixture of chloroform and methanol (1:1)
Application volume: 2 μ L
Developing solvent system: Chloroform, methanol, and a solution (1 in 30) of ammonium hydroxide (150:75:15). Saturate a paper-lined chromatographic chamber with this mixture.
- **B.** The retention time of the ofloxacin peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

ASSAY

- **PROCEDURE**
Mobile phase: Acetonitrile, 0.24% sodium dodecyl sulfate, and glacial acetic acid (400:580:20)
0.05 N hydrochloric acid: Add 4.0 mL of hydrochloric acid to 500 mL of water, dilute with water to 1000 mL, and mix.
System suitability solution: 0.1 mg/mL of USP Ofloxacin RS and 2.4 mg/mL of propylparaben in acetonitrile
Standard solution: 0.06 mg/mL of USP Ofloxacin RS in 0.05 N hydrochloric acid
Sample solution: 0.06 mg/mL of ofloxacin from Ophthalmic Solution in 0.05 N hydrochloric acid
Chromatographic system
(See Chromatography (621), System Suitability.)
Mode: LC
Detector: UV 294 nm
Column: 4.6-mm \times 25-cm; 5- μ m packing L1
Column temperature: 35°
Flow rate: 1.5 mL/min
Injection volume: 20 μ L
System suitability
Samples: System suitability solution and Standard solution

Suitability requirements

Resolution: NLT 2 between the propylparaben and ofloxacin peaks, System suitability solution
Tailing factor: NMT 3, Standard solution
Relative standard deviation: NMT 2.0%, Standard solution

Analysis

Samples: Standard solution and Sample solution
Calculate the percentage of the labeled amount of ofloxacin ($C_{18}H_{20}FN_3O_4$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak area of ofloxacin from the Sample solution
 r_S = peak area of ofloxacin from the Standard solution
 C_S = concentration of USP Ofloxacin RS in the Standard solution (mg/mL)
 C_U = nominal concentration of ofloxacin in the Sample solution (mg/mL)
Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **STERILITY TESTS (71):** It meets the requirements when tested as directed for Membrane Filtration in the test for Sterility of the Product to Be Examined.
- **PH (791):** 6.0–6.8

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Ofloxacin RS

Ofloxacin Tablets**DEFINITION**

Ofloxacin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ofloxacin ($C_{18}H_{20}FN_3O_4$).

IDENTIFICATION

- The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

ASSAY

- **PROCEDURE**
Solution A: Dissolve 2.72 mg/mL of monobasic potassium phosphate in water. Adjust with diluted phosphoric acid to a pH of 3.3 ± 0.1 .
Mobile phase: Acetonitrile and Solution A (3:22)
Diluent 1: Methanol and glacial acetic acid (3:1)
Diluent 2: Acetonitrile and water (1:9)
Standard stock solution: 1 mg/mL of USP Ofloxacin RS in Diluent 1
Standard solution: 20 μ g/mL of USP Ofloxacin RS from the Standard stock solution in Diluent 2
Sample solution: Transfer an equivalent of 100 mg of ofloxacin from powdered Tablets to a 100-mL volumetric flask. Add 70 mL of Diluent 1, and sonicate for 20 min. Dilute with Diluent 1 to volume. Pass a portion of this solution through a filter having a 0.45- μ m or finer porosity, and collect the filtrate. Dilute 2.0 mL of the filtrate with Diluent 2 to 100 mL.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 294 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of $C_{18}H_{20}FN_3O_4$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Ofloxacin RS in the *Standard solution* (mg/mL) C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard stock solution: 0.44 mg/mL of USP Ofloxacin RS in methanol

Standard solution: 8.8 µg/mL of USP Ofloxacin RS from the *Standard stock solution* diluted with *Medium*Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter. Dilute a portion of the filtrate with *Medium* to obtain a final theoretical concentration of 8.8 µg/mL, assuming complete dissolution of the label claim.**Spectrometric conditions**(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV-Vis

Analytical wavelength: 294 nm

Cell: 1 cm

Blank: *Medium***Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of $C_{18}H_{20}FN_3O_4$ dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (100/L)$$

 A_U = absorbance from the *Sample solution* A_S = absorbance from the *Standard solution* C_S = concentration of ofloxacin in the *Standard solution* (mg/mL) D = dilution factor of the *Sample solution* V = volume of *Medium*, 900 mL L = label claim of a Tablet (mg)Tolerances: NLT 80% (Q) of the labeled amount of $C_{18}H_{20}FN_3O_4$ is dissolved.• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**IMPURITIES****Organic Impurities**• **PROCEDURE 1**Phosphate buffer: 2.72 mg/mL of monobasic potassium phosphate in water. Adjust with diluted phosphoric acid to a pH of 3.3 ± 0.1 .

Solution A: Acetonitrile and Phosphate buffer (3:22)

Solution B: Acetonitrile and Phosphate buffer (3:2)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
8	100	0
25	40	60
26	100	0
40	100	0

Standard solution: 4 µg/mL of USP Ofloxacin RS in methanol

Sample solution: Transfer an equivalent of 100 mg of ofloxacin from powdered Tablets to a 100-mL volumetric flask. Add 70 mL of methanol, and sonicate for 20 min. Dilute with methanol to volume. Pass a portion of this solution through a filter having a 0.45-µm or finer porosity, discarding the first 5 mL. Use the filtrate.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 294 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 5.0%

AnalysisSamples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak response of the impurity from the *Sample solution* r_S = peak response of ofloxacin from the *Standard solution* C_S = concentration of USP Ofloxacin RS in the *Standard solution* (mg/mL) C_U = nominal concentration of ofloxacin in the *Sample solution* (mg/mL) F = relative response factor for each impurity (see *Impurity Table 1*)Acceptance criteria: See *Impurity Table 1*.**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity A ^a	0.5	1	0.3
Impurity B ^b	3.6	0.22	0.3
Any other impurity	—	1	0.2
All impurities	—	—	1.0

^a (2,3-Dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid).^b (9,10-Difluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid).**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

- **USP REFERENCE STANDARDS (11)**
USP Olanzapine RS

Hydrophilic Ointment

DEFINITION

Prepare Hydrophilic Ointment as follows.

Methylparaben	0.25 g
Propylparaben	0.15 g
Sodium Lauryl Sulfate	10 g
Propylene Glycol	120 g
Stearyl Alcohol	250 g
White Petrolatum	250 g
Purified Water	370 g
To make about	1000 g

Melt the *Stearyl Alcohol* and the *White Petrolatum* on a steam bath, and warm to about 75°. Add the other ingredients, previously dissolved in *Purified Water* and warmed to 75°, and stir the mixture until it congeals.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight containers.

White Ointment

DEFINITION

Prepare White Ointment as follows.

White Wax	50 g
White Petrolatum	950 g
To make	1000 g

Melt the *White Wax* in a suitable dish on a water bath, add the *White Petrolatum*, warm until liquefied, then discontinue the heating, and stir the mixture until it begins to congeal.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in well-closed containers.

Yellow Ointment

DEFINITION

Prepare Yellow Ointment as follows.

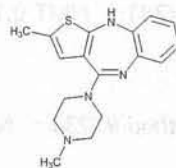
Yellow Wax	50 g
Petrolatum	950 g
To make	1000 g

Melt the *Yellow Wax* in a suitable dish on a steam bath, add the *Petrolatum*, warm until liquefied, then discontinue the heating, and stir the mixture until it begins to congeal.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in well-closed containers.

Olanzapine



$C_{17}H_{20}N_4S$ 312.43
10*H*-Thieno[2,3-*b*][1,5]benzodiazepine, 2-methyl-4-(4-methyl-1-piperazinyl)-;
2-Methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine [132539-06-1].

DEFINITION

Olanzapine contains NLT 98.0% and NMT 102.0% of olanzapine ($C_{17}H_{20}N_4S$), calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Buffer: Dissolve 6.9 g of monobasic sodium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 2.5, and dissolve 12 g of sodium dodecyl sulfate in the resulting solution.

Mobile phase: Acetonitrile and *Buffer* (47:53)

System suitability solution: 0.1 mg/mL of USP

Olanzapine RS and 0.01 mg/mL of USP Olanzapine Related Compound A RS in *Mobile phase*

Standard solution: 0.1 mg/mL of USP Olanzapine RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Olanzapine in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for olanzapine related compound A and olanzapine are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between olanzapine related compound A and olanzapine

Tailing factor: 0.8–1.5 for the olanzapine peak

Relative standard deviation: NMT 1.0% for the olanzapine peak

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of olanzapine ($C_{17}H_{20}N_4S$) in the portion of Olanzapine taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)

C_u = concentration of Olanzapine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous, solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1-

Jan-2018)

- **ORGANIC IMPURITIES**

Buffer: Dissolve 13 g of sodium dodecyl sulfate in 1500 mL of water. Add 5 mL of phosphoric acid, and adjust with a sodium hydroxide solution to a pH of 2.5.

Solution A: Acetonitrile and Buffer (48:52)

Solution B: Acetonitrile and Buffer (70:30)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
20	0	100
25	0	100
27	100	0
35	100	0

Eдетate disodium solution: 37 mg/L of edetate disodium in Buffer

Diluent: Acetonitrile and Eдетate disodium solution (40:60)

System suitability solution: 20 µg/mL of USP Olanzapine RS and 2 µg/mL each of USP Olanzapine Related Compound A RS and USP Olanzapine Related Compound B RS in Diluent

Standard solution: 2 µg/mL of USP Olanzapine RS in Diluent

Sample solution: 0.4 mg/mL of Olanzapine in Diluent

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Temperatures

Column: 35°

Sample: 5°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: System suitability solution

[NOTE—Identify the peaks using the Relative Retention Time values given in Table 2.]

Suitability requirements

Resolution: NLT 3.0 between olanzapine related compound A and olanzapine

Tailing factor: NMT 1.5 for the olanzapine peak

Relative standard deviation: NMT 2.0% from four replicate injections for the olanzapine peak

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Olanzapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the Sample solution

r_S = peak response of olanzapine from the Standard solution

C_S = concentration of USP Olanzapine RS in the Standard solution (mg/mL)

C_U = concentration of Olanzapine in the Sample solution (mg/mL)

F = relative response factor for each impurity from Table 2

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olanzapine related compound B ^a	0.3	2.3	0.10
Olanzapine related compound A ^b	0.8	2.3	0.10
Olanzapine	1.0	—	—
Chloromethyl olanzapinium chloride ^c (if present)	1.1	1.0	0.15
Any individual, unspecified impurity	—	—	0.10
Total impurities	—	—	0.4

^a 2-Methyl-10H-thieno-[2,3-b][1,5]benzodiazepin-4[5H]-one.

^b 5-Methyl-2-((2-nitrophenyl)amino)-3-thiophenecarbonitrile.

^c 1-Chloromethyl-1-methyl-4-(2-methyl-10H-benzo[b]thieno[2,3-e][1,4]diazepin-4-yl)piperazin-1-ium chloride.

SPECIFIC TESTS

- **WATER DETERMINATION, Method I** (921)

[NOTE—A suitable solvent system for water determination in ketones and aldehydes (e.g., Hydranal composite 5K-working medium K or Aquastar composite 5K-solvent KC or equivalent) is recommended.]

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.

- **USP REFERENCE STANDARDS** (11)

USP Olanzapine RS

USP Olanzapine Related Compound A RS

5-Methyl-2-((2-nitrophenyl)amino)-

3-thiophenecarbonitrile.

$C_{12}H_9N_3O_2S$ 259.28

USP Olanzapine Related Compound B RS

2-Methyl-10H-thieno-[2,3-b][1,5]benzodiazepin-4[5H]-one.

$C_{12}H_{10}N_2OS$ 230.29

Olanzapine Tablets

DEFINITION

Olanzapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$).

IDENTIFICATION

- **INFRARED ABSORPTION** (197K)

Standard: Dissolve 10 mg of USP Olanzapine RS in 10 mL of chloroform. Evaporate to dryness on a water bath maintained at 55°. Use about 2 mg of the residue to prepare a potassium bromide pellet.

Sample: Crush NLT 5 Tablets, and transfer the powder equivalent to 30 mg of olanzapine to a suitable container. Add 30 mL of chloroform, and sonicate for 15 min to dissolve. Pass through a suitable filter, and evaporate the filtrate to dryness on a water bath maintained at 55°. Use about 2 mg of the residue to prepare a potassium bromide pellet.

Acceptance criteria: Meet the requirements

ASSAY

PROCEDURE

[NOTE—A few drops of acetonitrile, not to exceed 5% of the final volume, may be added to the *Standard solution* and *Sample solution* before final dilution to reduce foaming.]

Buffer 1: 6.9 g/L of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.5.

Buffer 2: 12 g/L of sodium dodecyl sulfate in *Buffer 1*

Mobile phase: Acetonitrile and *Buffer 2* (1:1)

System suitability solution: 0.1 mg/mL of USP Olanzapine RS and 0.01 mg/mL of USP Olanzapine Related Compound A RS in *Mobile phase*

Standard solution: 0.1 mg/mL of USP Olanzapine RS in *Mobile phase*

Sample solution: Transfer a known quantity of Tablets (NLT 5), equivalent to NLT 25 mg of olanzapine, to a suitable volumetric flask. Dilute with *Mobile phase* to volume, mix, and sonicate for 10 min. Centrifuge a portion of this solution, and dilute the clear supernatant with *Mobile phase* to obtain a solution containing about 0.1 mg/mL of olanzapine. [NOTE—Agitation of the flask may be necessary before sonication to prevent Tablets from adhering to the flask, making disintegration and dissolution difficult.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for olanzapine related compound A and olanzapine are 0.89 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between olanzapine and olanzapine related compound A, *System suitability solution*

Tailing factor: NMT 1.8, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of olanzapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Test 1

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Mobile phase: 10 g/L of ammonium acetate in a mixture of methanol and water (2:3). Adjust with hydrochloric acid to a pH of 4.0.

Standard solution: ($L/1000$) mg/mL of USP Olanzapine RS in *Medium*, where L is the label claim in mg/Tablet. Transfer 5.0 mL of this solution to a tube, and add 2.0 mL of *Mobile phase*.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size. Transfer 5.0 mL of the filtrate to a tube, and add 2.0 mL of *Mobile phase*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 15-cm; 5-μm packing L10

Flow rate: 1.5 mL/min

Injection volume: 50 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 20 min

Mobile phase: 10 g/L of ammonium acetate in a mixture of methanol and water (2:3). Adjust with hydrochloric acid to a pH of 4.0. Pass through a suitable filter of 0.45-μm pore size.

Standard stock solution: 0.28 mg/mL of USP

Olanzapine RS prepared as follows. Transfer a suitable amount of USP Olanzapine RS to a suitable volumetric flask. Add about 8% of the final flask volume of acetonitrile. Sonicate to dissolve the Reference Standard. Dilute with *Medium* to volume.

Standard solution: ($L/900$) mg/mL of USP Olanzapine RS in *Medium* from *Standard stock solution*, where L is the label claim in mg/Tablet. Transfer 5.0 mL of this solution to a tube, and add 2.0 mL of *Mobile phase*.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size. Transfer 5.0 mL of the filtrate to a tube, and add 2.0 mL of *Mobile phase*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 15-cm; 5-μm packing L11

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Olanzapine RS in the Standard solution (mg/mL)

V = volume of Medium, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

[NOTE—A few drops of acetonitrile, not to exceed 5% of the final volume, may be added to the Standard solution and Sample solution before final dilution to reduce foaming.]

Buffer 1: 3.3 mL/L of phosphoric acid. Adjust with 50% sodium hydroxide to a pH of 2.5.

Buffer 2: 8.7 g/L of sodium dodecyl sulfate in Buffer 1

Buffer 3: 18.6 mg/L of edetate disodium (EDTA) in Buffer 2

Solution A: Acetonitrile and Buffer 2 (12:13)

Solution B: Acetonitrile and Buffer 2 (7:3)

Diluent: Acetonitrile and Buffer 3 (2:3)

System suitability solution: 20 µg/mL of USP

Olanzapine RS and 2 µg/mL each of USP Olanzapine Related Compound B RS and USP Olanzapine Related Compound C RS in Diluent

Standard solution: 0.002 mg/mL of USP Olanzapine RS in Diluent

Sensitivity solution: 0.4 µg/mL of USP Olanzapine RS in Diluent from the Standard solution

Sample solution: Nominally 0.375–0.500 mg/mL of olanzapine from a suitable number of Tablets prepared as follows. Transfer a known quantity of Tablets to a suitable volumetric flask, and dilute with Diluent to volume. Centrifuge a portion of this solution, and use the supernatant. [NOTE—Immediate agitation of the flask may be necessary to prevent Tablets from adhering to the flask, making dissolution and disintegration difficult. **[CAUTION—Do not sonicate.]** The Sample solution is stable for 12 h at room temperature and 48 h if refrigerated.]

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
20	0	100
25	0	100
27	100	0
35	100	0

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: System suitability solution, Standard solution, and Sensitivity solution

Suitability requirements

Resolution: NLT 3.0 between olanzapine and olanzapine related compound C, System suitability solution

Tailing factor: NMT 1.5 for the olanzapine peak, System suitability solution

Relative standard deviation: NMT 2.0%, Standard solution

Signal-to-noise ratio: NLT 10, Sensitivity solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Olanzapine RS in the Standard solution (mg/mL)

C_U = nominal concentration of olanzapine in the Sample solution (mg/mL)

F = relative response factor for each impurity listed in Table 2

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olanzapine lactam ^a	0.26	1.0	0.50
Olanzapine related compound B	0.30	2.3	0.50
Olanzapine thiolactam ^b	0.34	1.0	0.50
Olanzapine related compound C	0.83	0.76	0.50
Olanzapine	1.0	—	—
Any individual unspecified degradation product	—	1.0	0.20
Total impurities	—	—	1.5

^a (Z)-4-(4-Methylpiperazin-1-yl)-3-(2-oxopropylidene)-1H-benzo[b][1,4]diazepin-2(3H)-one.

^b (Z)-1-[4-(4-Methylpiperazin-1-yl)-2-thioxo-1H-benzo[b][1,4]diazepin-3(2H)-ylidene]propan-2-one.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

- **LABELING:** When more than one Dissolution test is given, the labeling states the Dissolution test used only if Test 1 is not used.

• USP REFERENCE STANDARDS (11)

USP Olanzapine RS

USP Olanzapine Related Compound A RS

5-Methyl-2-((2-nitrophenyl)amino)-3-thiophenecarbonitrile.

$C_{12}H_9N_3O_2S$ 259.28

USP Olanzapine Related Compound B RS

2-Methyl-10H-thieno-[2,3-b][1,5]benzodiazepin-4[5H]-one.

$C_{12}H_{10}N_2OS$ 230.29

USP Olanzapine Related Compound C RS

2-Methyl-4-(4-methylpiperazin-1-yl)-10H-benzo[b]thieno-[2,3-e][1,4]diazepine 4'-N-oxide.

$C_{17}H_{20}N_4OS$ 328.43

Olanzapine and Fluoxetine Capsules

DEFINITION

Olanzapine and Fluoxetine Capsules contain an amount of Olanzapine and Fluoxetine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% each of the labeled

amount of olanzapine ($C_{17}H_{20}N_4S$) and fluoxetine ($C_{17}H_{18}F_3NO$).

IDENTIFICATION

- A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Buffer: 37 mg/L of disodium ethylenediaminetetraacetate in water. Add 3.3 mL of phosphoric acid, and adjust with 50% sodium hydroxide to a pH of 2.5. Dissolve 8.7 g of sodium dodecyl sulfate in the resulting solution.

Mobile phase: Acetonitrile and *Buffer* (1:1)

Standard solution: 0.12 mg/mL of USP Olanzapine RS and 0.45 mg/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*

Sample solution: Nominally 0.06–0.18 mg/mL of olanzapine and 0.25–0.5 mg/mL of fluoxetine in *Mobile phase* from a counted number of Capsules prepared as follows. Place the Capsules (including shells) into a suitable volumetric flask and fill to about half volume with *Mobile phase*. Mix for NLT 30 min. If disintegration is incomplete, sonicate for NMT 5 min. Dilute with *Mobile phase* to volume, mix, and filter or centrifuge.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 227 nm

Column: 4.6-mm \times 7.5-cm; 3.5- μ m packing L7

Column temperature: 40°

Flow rate: 2 mL/min

Injection volume: 10 μ L

Run time: 2.5 times the retention time of olanzapine

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for olanzapine and fluoxetine are 1.0 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 2.0 between olanzapine and fluoxetine

Relative standard deviation: NMT 2.0% for the olanzapine and fluoxetine peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of olanzapine from the *Sample solution*

r_S = peak response of olanzapine from the *Standard solution*

C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of olanzapine in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of fluoxetine from the *Sample solution*

r_S = peak response of fluoxetine from the *Standard solution*

C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of fluoxetine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of fluoxetine, 309.33

M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL, deaerated. [NOTE—Helium sparging recommended.]

Apparatus 2: 50 rpm, with 3-prong sinkers

Time: 30 min for both olanzapine and fluoxetine

Standard solution: USP Olanzapine RS and USP Fluoxetine Hydrochloride RS in *Medium* to obtain a final concentration of (L/1000) mg/mL each, where L is the Capsule label claim, in mg

Sample solution: Pass a portion of the solution through a suitable filter of 0.45- μ m pore size.

Buffer, Mobile phase, Chromatographic system, System suitability, and Analysis: Proceed as directed in the *Assay*.

Calculate the percentage of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of olanzapine from the *Sample solution*

r_S = peak response of olanzapine from the *Standard solution*

C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)

L = label claim for olanzapine (mg/Capsule)

V = volume of *Medium*, 900 mL

Calculate the percentage of the labeled amount of fluoxetine ($C_{17}H_{18}F_3NO$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

r_U = peak response of fluoxetine from the *Sample solution*

r_S = peak response of fluoxetine from the *Standard solution*

C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL)

L = label claim for fluoxetine (mg/Capsule)

M_{r1} = molecular weight of fluoxetine, 309.33

M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amounts of olanzapine ($C_{17}H_{20}N_4S$) and fluoxetine ($C_{17}H_{18}F_3NO$) are dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

ORGANIC IMPURITIES

Buffer and Mobile phase: Proceed as directed in the *Assay*.

System suitability solution: 0.1 mg/mL of USP Olanzapine RS, 0.11 mg/mL of USP Fluoxetine Hydrochloride RS, and 0.002 mg/mL each of α [(2-methylamino)ethyl] benzyl alcohol, trifluoro-*p*-cresol, USP Fluoxetine Related Compound B RS, and USP Olanzapine Related Compound B RS in *Mobile phase*

Standard solution: 2 μ g/mL of USP Olanzapine RS and 8 μ g/mL of USP Fluoxetine Hydrochloride RS in *Mobile phase*

Sample solution: Empty the Capsules, and combine the contents in a suitable container. The contents of the Capsules may be powdered in a mortar, if necessary. Transfer an amount of the sample to a suitable volumetric flask to obtain nominally 0.2 mg/mL of olanzapine and 0.27–1.7 mg/mL of fluoxetine and fill to about 70% volume with *Mobile phase*. Mix for about 5 min. Dilute with *Mobile phase* to volume, mix, and filter or centrifuge.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 215 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L7**Temperatures****Column:** 35°**Autosampler:** 5°**Flow rate:** 1.5 mL/min**Injection volume:** 50 μL**Run time:** 1.5 times the retention time of fluoxetine**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements**[NOTE—Identify the peaks using *Table 1*.]**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
α[(2-Methylamino)ethyl] benzyl alcohol ^a	0.22	—	0.25
Olanzapine related compound B ^b	0.24	1.73	0.20
Trifluoro- <i>p</i> -cresol ^a	0.30	—	0.25
Fluoxetine related compound B ^a	0.31	—	0.25
Olanzapine	0.63	—	—
Fluoxetine	1.0	—	—
Any individual fluoxetine related degradation product	—	—	0.25
Any individual olanzapine related degradation product ^c	—	1.0	0.20
Total impurities (fluoxetine related) ^d	—	—	0.40
Total impurities (olanzapine related) ^e	—	—	1.5

^a Fluoxetine related degradation product.^b Olanzapine related degradation product.^c Any other degradation product with a relative retention time <0.63 except fluoxetine related degradation products, and any degradation product with a relative retention time >1.0.^d Sum of all specified fluoxetine related degradation products and any other fluoxetine related degradation product with relative retention times of 0.63 and 1.0.^e Sum of all specified olanzapine degradation products, any other degradation product with a relative retention time <0.63 except fluoxetine related degradation products, and any degradation product with relative retention time >1.0.**Resolution:** NLT 1.9 between α[(2-methylamino)ethyl] benzyl alcohol and olanzapine related compound B, *System suitability solution***Tailing factor:** NMT 1.8 for olanzapine and fluoxetine, *System suitability solution* and *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

[NOTE—Peaks eluting before a relative retention time of 0.63 and after a relative retention time of 1.0, excluding any peak with relative retention times of 0.22, 0.30, and 0.31, are olanzapine related degradation products.]

Calculate the percentage of each olanzapine related degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak response of each individual impurity from the *Sample solution* r_S = peak response of olanzapine from the *Standard solution* C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL) C_U = nominal concentration of olanzapine in the *Sample solution* (mg/mL) F = relative response factor (see *Table 1*)

[NOTE—Peaks eluting at relative retention times of 0.22, 0.30, and 0.31, and any peaks between a relative retention time of 0.63 and 1.0, are fluoxetine related degradation products.]

Calculate the percentage of each fluoxetine related degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of each individual impurity from the *Sample solution* r_S = peak response of fluoxetine from the *Standard solution* C_S = concentration of USP Fluoxetine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = nominal concentration of fluoxetine in the *Sample solution* (mg/mL) M_{r1} = molecular weight of fluoxetine, 309.33 M_{r2} = molecular weight of fluoxetine hydrochloride, 345.79Acceptance criteria: See *Table 1*.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.• **USP REFERENCE STANDARDS (11)**

USP Fluoxetine Hydrochloride RS

Benzenepropanamine, *N*-methyl-γ-[4-(trifluoromethyl)phenoxy]-, hydrochloride, ±
 $C_{17}H_{18}F_3NO \cdot HCl$ 345.79

USP Fluoxetine Related Compound B RS

N-Methyl-3-phenylpropylamine. $C_{10}H_{15}N$ 149.23

USP Olanzapine RS

10*H*-Thieno[2,3-*b*][1,5]benzodiazepine, 2-methyl-4-(4-methyl-1-piperazinyl). $C_{17}H_{20}N_4S$ 312.43

USP Olanzapine Related Compound B RS

2-Methyl-10*H*-thieno-[2,3-*b*][1,5]benzodiazepin-4[5*H*]-one. $C_{12}H_{10}N_2OS$ 230.29**Olanzapine Orally Disintegrating Tablets****DEFINITION**Olanzapine Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of olanzapine ($C_{17}H_{20}N_4S$).**IDENTIFICATION**• **A. INFRARED ABSORPTION (197K)****Standard:** Add 25 mg of USP Olanzapine RS to a suitable container containing 10 mL of water. Centrifuge for 5 min, and discard the supernatant. Dry the olanzapine under vacuum for 1 h at 60°.**Sample:** Place 1 Tablet in 10 mL of water in a centrifuge tube. Centrifuge for 5 min. Wash the precipitate with 5 mL of water, and centrifuge for 5 min. Discard the water, and repeat the process a third time. Dry the olanzapine under vacuum for 1 h at 60°.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Solution A: Acetonitrile and water (20:80). Add 2 mL of perchloric acid to each L of the mixture.

Solution B: Acetonitrile and water (60:40). Add 2 mL of perchloric acid to each L of the mixture.

Diluent: Acetonitrile and water (35:65)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	87	13
10	87	13
20	0	100
26	0	100
26.1	87	13
36	87	13

System suitability solution: 0.1 mg/mL of USP Olanzapine RS and 10 µg/mL of USP Olanzapine Related Compound C RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Olanzapine RS in *Diluent*

Sample stock solution: 0.5–0.6 mg/mL of olanzapine from NLT 10 Tablets, prepared as follows. Transfer the Tablets to a suitable volumetric flask. Add *Diluent* to fill about 80% of the flask volume. Shake mechanically for 10 min, and dilute with *Diluent* to volume.

Sample solution: Nominally, 0.1–0.12 mg/mL of olanzapine from the *Sample stock solution* in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the olanzapine and olanzapine related compound C peaks, *System suitability solution*

Tailing factor: NMT 2.0 for the olanzapine peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of olanzapine (C₁₇H₂₀N₄S) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of olanzapine from the *Sample solution*

r_S = peak response of olanzapine from the *Standard solution*

C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of olanzapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISINTEGRATION (701)

Test 1: NMT 10 s for each of 6 units

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Disintegration Test 2*

Acceptance criteria: NMT 30 s

DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 10 min

Standard solution: (L/900) mg/mL of USP Olanzapine RS in *Medium*

Sample solution: A portion of the solution under test may be either centrifuged or filtered through a suitable membrane filter of 0.45-µm pore size, discarding the first few mL. The filtrate or centrifugate may be suitably diluted.

Determine the amount of olanzapine (C₁₇H₂₀N₄S) dissolved by using either the *Spectrometric procedure* or *Chromatographic procedure* described below:

Spectrometric procedure

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV-Vis

Analytical wavelength: 344 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of olanzapine (C₁₇H₂₀N₄S) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Olanzapine RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Chromatographic procedure

Buffer: 6.8 g/L of sodium acetate trihydrate in water.

Adjust with glacial acetic acid to a pH of 5.0.

Mobile phase: Acetonitrile and *Buffer* (25:75)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 5-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of olanzapine (C₁₇H₂₀N₄S) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of olanzapine from the *Sample solution*

r_S = peak response of olanzapine from the *Standard solution*

C_S = concentration of USP Olanzapine RS (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of olanzapine (C₁₇H₂₀N₄S) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES**• ORGANIC IMPURITIES**

Solution A: Acetonitrile and water (20:80). Add 2 mL of perchloric acid to each L of the mixture.

Solution B: Acetonitrile and water (60:40). Add 2 mL of perchloric acid to each L of the mixture.

Diluent: Acetonitrile and water (35:65)

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	93	7
13	93	7
28	0	100
33	0	100
33.1	93	7
44	93	7

System suitability solution: 0.1 mg/mL of USP Olanzapine RS and 7 µg/mL of USP Olanzapine Related Compound C RS in *Diluent*

Standard solution: 1.5 µg/mL of USP Olanzapine RS in *Solution A*

Sensitivity solution: 0.15 µg/mL of USP Olanzapine RS from the *Standard solution* in *Diluent*

Sample solution: Nominally, 0.3 mg/mL of olanzapine from NLT 10 Tablets, prepared as follows. Transfer the Tablets to a suitable volumetric flask. Add *Diluent* to fill about 80% of the flask volume. Shake mechanically for 25 min, and dilute with *Diluent* to volume. Dilute a portion of this solution with water to volume. Store the solution at 4°. Use within 7 h.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Sample temperature: 4°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 2.0 between the olanzapine and olanzapine related compound C peaks, *System suitability solution*

Tailing factor: NMT 2.0 for the olanzapine peak, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual specified impurity and any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each individual specified impurity from the *Sample solution*

r_s = peak response of olanzapine from the *Standard solution*

C_s = concentration of olanzapine in the *Standard solution* (mg/mL)

C_u = nominal concentration of olanzapine in the *Sample solution* (mg/mL)

F = relative response factor (see Table 3)

Acceptance criteria: See Table 3. [NOTE—Disregard any peak less than 0.05%.]

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olanzapine lactam ^a	0.48	1.9	0.50
Olanzapine thio-lactam ^b	0.75	1.0	0.50
Olanzapine	1.0	—	—
Olanzapine related compound C	1.2	0.79	0.50
Olanzapine related compound B ^c	2.1	1.9	0.50
Any individual unspecified degradation product	—	1.0	0.20
Total impurities	—	—	1.5

^a (Z)-4-(4-Methylpiperazin-1-yl)-3-(2-oxopropylidene)-1H-benzo[b][1,4]diazepin-2(3H)-one.

^b (Z)-1-[4-(4-Methylpiperazin-1-yl)-2-thioxo-1H-benzo[b][1,4]diazepin-3(2H)-ylidene]propan-2-one.

^c 2-Methyl-5,10-dihydro-4H-benzo[b]thieno[2,3-e][1,4]diazepin-4-one.

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in well-closed containers. Store at controlled room temperature.

• LABELING: When more than one *Disintegration* test is given, the labeling states the *Disintegration* test used only if *Test 1* is not used. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

• USP REFERENCE STANDARDS (11)

USP Olanzapine RS

USP Olanzapine Related Compound C RS

2-Methyl-4-(4-methylpiperazin-1-yl)-10H-benzo[b]thieno[2,3-e][1,4]diazepine 4'-N-oxide.

C₁₇H₂₀N₄O₅ 328.43

Oleovitamin A and D**DEFINITION**

Oleovitamin A and D is a solution of vitamin A and vitamin D in fish liver oil or in an edible vegetable oil. The vitamin D is present as ergocalciferol or cholecalciferol obtained by the activation of ergosterol or 7-dehydrocholesterol, or from natural sources. Oleovitamin A and D contains NLT 90.0% of the labeled amounts of vitamins A and D.

ASSAY**• A. VITAMIN A ASSAY (571)**

Analysis: The acceptance criteria may be met by following any one of the specified procedures described in the *Chemical Methods* or *Chromatographic Methods* in the chapter. The method and procedure used should be stated in the labeling.

Acceptance criteria: NLT 90.0% of the labeled amount

• B. VITAMIN D ASSAY (581)

Analysis: The acceptance criteria may be met by following any one of the specified procedures described in the *Chromatographic Methods* in the chapter. The procedure used should be stated in the labeling.

Acceptance criteria: NLT 90.0% of the labeled amount

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in tight containers, protected from light and air, preferably under an atmosphere of an inert gas. Store in a dry place.

• LABELING: Label it to indicate the content of vitamin A in terms of retinol/g. The vitamin A content may also be expressed in USP Vitamin A Units/g. Label it to show

whether it contains ergocalciferol, cholecalciferol, or vitamin D from a natural source. Label it to indicate the vitamin D content, in μg , of ergocalciferol or cholecalciferol/g. Its vitamin D content may be expressed also in USP Vitamin D Units/g.

- **USP REFERENCE STANDARDS** (11)
 - USP Cholecalciferol RS
 - USP Ergocalciferol RS
 - USP Retinyl Acetate RS
 - USP Retinyl Palmitate RS

Oleovitamin A and D Capsules

DEFINITION

Oleovitamin A and D Capsules contain NLT 90.0% of the labeled amounts of vitamins A and D. The oil in Oleovitamin A and D Capsules is a solution of vitamin A and vitamin D in fish liver oil or in an edible vegetable oil. The vitamin D is present as ergocalciferol or cholecalciferol obtained by the activation of ergosterol or 7-dehydrocholesterol or from natural sources.

ASSAY

- **VITAMIN A ASSAY** (571)

Analysis: Use the appropriate *Chemical Method* or *Chromatographic Method* as described in the chapter.

Acceptance criteria: NLT 90.0% of the labeled amount
- **VITAMIN D ASSAY** (581)

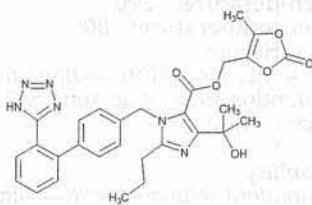
Analysis: Use the appropriate *Chromatographic Method* as described in the chapter.

Acceptance criteria: NLT 90.0% of the labeled amount

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a dry place.
- **LABELING:** Label the Capsules to indicate the content, in mg, of vitamin A in each Capsule. The vitamin A content in each Capsule may be expressed also in USP Vitamin A Units. Label the Capsules to show whether they contain ergocalciferol, cholecalciferol, or vitamin D from a natural source. Label the Capsules to indicate also the vitamin D content, in μg , in each Capsule. The vitamin D content may be expressed also in USP Vitamin D Units in each Capsule.
- **USP REFERENCE STANDARDS** (11)
 - USP Cholecalciferol RS
 - USP Ergocalciferol RS
 - USP Retinyl Acetate RS
 - USP Retinyl Palmitate RS

Olmesartan Medoxomil



$\text{C}_{29}\text{H}_{30}\text{N}_6\text{O}_6$ 558.59
 1*H*-Imidazole-5-carboxylic acid, 4-[(1-hydroxy-1-methylethyl)-2-propyl-1-[[2'-(1*H*-tetrazol-5-yl) [1,1'-biphenyl]-4-yl]methyl]-, (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester; (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-4-(2-hydroxypropan-2-yl)-2-propyl-1*H*-imidazole-5-carboxylate [144689-63-4].

DEFINITION

Olmesartan Medoxomil contains NLT 98.5% and NMT 101.5% of $\text{C}_{29}\text{H}_{30}\text{N}_6\text{O}_6$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The ratio of the retention time of the major peak to that of the internal standard of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

- **PROCEDURE**

[NOTE—The *Standard solution* and *Sample solution* are stable for 24 h at 5°.]

Diluted phosphoric acid: 0.2% phosphoric acid

Buffer: 0.015 M monobasic potassium phosphate. Adjust the solution with *Diluted phosphoric acid* (w/v) to a pH of 3.4.

Mobile phase: Acetonitrile and *Buffer* (17:33)

Diluent 1: Acetonitrile and water (4:1)

Diluent 2: Acetonitrile and water (2:3)

Internal standard solution: 0.5 mg/mL of 4-hydroxybenzoic acid isobutyl ester in *Diluent 2*. [NOTE—This solution is stable for 1 month at room temperature.]

Standard stock solution: 1 mg/mL of USP Olmesartan Medoxomil RS in *Diluent 1*

Standard solution: 0.05 mg/mL of USP Olmesartan Medoxomil RS from the *Standard stock solution* and 0.025 mg/mL of *p*-hydroxybenzoic acid isobutyl ester from the *Internal standard solution* in *Diluent 2*

Sample stock solution: 1 mg/mL of Olmesartan Medoxomil in *Diluent 1*

Sample solution: 0.05 mg/mL of Olmesartan Medoxomil from the *Sample stock solution* and 0.025 mg/mL of *p*-hydroxybenzoic acid isobutyl ester from the *Internal standard solution* in *Diluent 2*

Chromatographic system
 (See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm \times 15-cm; 5- μm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection size: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 4 between olmesartan medoxomil and *p*-hydroxybenzoic acid isobutyl ester

Relative standard deviation: NMT 0.5% for the peak ratio of olmesartan medoxomil and the internal standard

Analysis

Samples: *Standard solution* and *Sample solution*
 Calculate the percentage of olmesartan medoxomil in the portion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

- R_U = ratio of the peak areas of olmesartan medoxomil and *p*-hydroxybenzoic acid isobutyl ester from the *Sample solution*
- R_S = ratio of the peak areas of olmesartan medoxomil and *p*-hydroxybenzoic acid isobutyl ester from the *Standard solution*
- C_S = concentration of USP Olmesartan Medoxomil RS in the *Standard solution* (mg/mL)
- C_U = concentration of Olmesartan Medoxomil in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the anhydrous and solvent-free basis

IMPURITIES**Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%. [NOTE—The ignition temperature range is 450° to 550°.]

Delete the following:

- **HEAVY METALS**, Method II (231): NMT 10 ppm. (Official 1-Jan-2018)

Organic Impurities• **PROCEDURE**

Buffer: Prepare as directed in the Assay.

Solution A: Acetonitrile and Buffer (1:4)

Solution B: Acetonitrile and Buffer (4:1)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	75	25
10	75	25
35	0	100
45	0	100

System suitability solution: 0.01 mg/mL each of USP Olmesartan Medoxomil RS and USP Olmesartan Medoxomil Related Compound A RS in acetonitrile

Standard solution: 0.01 mg/mL of USP Olmesartan Medoxomil RS in acetonitrile

Sample solution: 1 mg/mL of Olmesartan Medoxomil in acetonitrile

Chromatographic system

(See Chromatography (621), System Suitability.)

[NOTE—A guard column of 4.6-mm × 5-cm of packing L7 may be used.]

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 10-cm; 3.5-μm packing L7

Column temperature: 40°

Flow rate: 1 mL/min

Injection size: 10 μL

System suitability**Suitability requirements**

Sample: System suitability solution

Resolution: NLT 5 between olmesartan medoxomil and olmesartan medoxomil related compound A

Relative standard deviation: NMT 2.0% for the olmesartan medoxomil peak

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Olmesartan Medoxomil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the Sample solution

r_S = peak response of olmesartan medoxomil from the Standard solution

C_S = concentration of USP Olmesartan Medoxomil RS in the Standard solution (mg/mL)

C_U = concentration of Olmesartan Medoxomil in the Sample solution (mg/mL)

F = relative response factor (see the Impurity Table)

Acceptance criteria

Individual impurities: See the Impurity Table.

Total impurities: NMT 1.3%. [NOTE—Disregard any peak below 0.05%.]

Impurity Table

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olmesartan ^a	0.2	1.0	0.5
Olmesartan medoxomil related compound A ^b	0.7	1.6	0.1
Olmesartan medoxomil	1.0	1.0	—
Olefinic impurity ^c	1.6	1.0	0.6
N-alkyl impurity ^d	3.4	0.7	0.1
Any other individual unidentified impurity	—	1.0	0.1

^a 1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-4-(2-hydroxypropan-2-yl)-2-propyl-1H-imidazole-5-carboxylic acid.

^b 1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-4,4-dimethyl-2-propyl-1H-furo[3,4-d]imidazol-6(4H)-one.

^c (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-4-(prop-1-en-2-yl)-2-propyl-1H-imidazole-5-carboxylate.

^d (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-[(2'-(2-trityl-2H-tetrazol-5-yl)biphenyl-4-yl)methyl]-1H-imidazole-5-carboxylate.

SPECIFIC TESTS• **LIMIT OF ACETONE (IF PRESENT)**

Internal standard solution: 1% solution of 1-butanol in dimethyl sulfoxide. [NOTE—This solution is stable for 1 month at room temperature.]

Standard solution: 0.37 μL/mL of acetone and 2 μL/mL of 1-butanol from the Internal standard solution in dimethylsulfoxide. [NOTE—This solution is stable for 8 h at room temperature.]

Sample solution: 25 mg/mL of Olmesartan Medoxomil and 2 μL/mL of 1-butanol from the Internal standard solution in dimethylsulfoxide. [NOTE—This solution is stable for 8 h at room temperature.]

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: GC

Detector: Flame ionization

Column: 30-m × 0.53-mm column bonded with a 1-μm film of phase G14

Column temperature: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	5
50	10	180	5

Injection port temperature: 200°

Detector temperature: 200°

Autosampler temperature: 80°

Carrier gas: Helium

Flow rate: 4 mL/min. [NOTE—Adjust the flow rate so that the retention time of acetone is 2.5 min.]

Injection size: 1 mL

Split ratio: 5:1

System suitability

Sample: Standard solution. [NOTE—Allow the samples to stand for 30 min in the autosampler at 80°.]

Suitability requirements

Resolution: NLT 60 between the acetone and 1-butanol peaks

Relative standard deviation: NMT 5.0% for the peak area ratio of acetone and 1-butanol

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetone in the portion of Olmesartan Medoxomil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

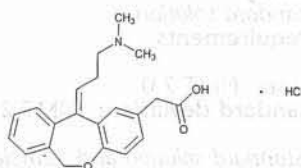
- r_U = peak response of acetone from the *Sample solution*
 r_S = peak response of acetone from the *Standard solution*
 C_S = concentration of acetone in the *Standard solution* (mg/mL)
 C_U = concentration of Olmesartan Medoxomil in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.6%

- **WATER DETERMINATION, Method 1c (921):** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protect from moisture, and store below 25°.
- **USP REFERENCE STANDARDS (11)**
 USP Olmesartan Medoxomil RS
 USP Olmesartan Medoxomil Related Compound A RS
 1-[[2'-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl]-4,4-dimethyl-2-propyl-1*H*-furo[3,4-*d*]imidazol-6(4*H*)-one.
 $C_{24}H_{24}N_6O_2$ 428.49

Olopatadine Hydrochloride

$C_{21}H_{23}NO_3 \cdot HCl$ 373.87
 Dibenz[*b,e*]oxepin-2-acetic acid, 11-[3-(dimethylamino)propylidene]-6,11-dihydro-, hydrochloride, (*Z*);
 11-[(*Z*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenzo[*b,e*]oxepin-2-acetic acid, hydrochloride [140462-76-6].

DEFINITION

Olopatadine Hydrochloride contains NLT 98.0% and NMT 102.0% of olopatadine hydrochloride ($C_{21}H_{23}NO_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION**Change to read:**

- **A. INFRARED ABSORPTION** Δ (197): [NOTE—Methods described in (197K) or (197A) may be used.] Δ USP40
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL (191), Chloride:** Meets the requirements

ASSAY**Change to read:**

- **PROCEDURE**
 Protect all solutions containing olopatadine hydrochloride from light.
Buffer: Dissolve 13.6 g of monobasic potassium phosphate in 1 L of water, add 1 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Buffer* (28:72)

Standard solution: 0.1 mg/mL of USP Olopatadine Hydrochloride RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Olopatadine Hydrochloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 299 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Flow rate: 1 mL/min

Injection volume: 30 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Δ USP40

Tailing factor: NMT 2.0

Relative standard deviation: NMT Δ 0.73% Δ USP40

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of olopatadine hydrochloride ($C_{21}H_{23}NO_3 \cdot HCl$) in the portion of Olopatadine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Olopatadine Hydrochloride RS in the *Standard solution* (mg/mL)
 C_U = concentration of Olopatadine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

Delete the following:

- **HEAVY METALS (231), Method II:** NMT 10 ppm (Official 1, Jan-2018)

Change to read:**• ORGANIC IMPURITIES**

Protect all solutions containing olopatadine hydrochloride from light.

Mobile phase: Prepare as directed in the *Assay*.

System suitability solution: 0.2 mg/mL of USP

Olopatadine Hydrochloride RS and 0.02 mg/mL of USP

Olopatadine Related Compound B RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Olopatadine Hydrochloride in *Mobile phase*

Blank solution: *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 299 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Flow rate: 1 mL/min

Injection volume: 30 μ L

Run time: At least 2.5 times the retention time of the major peak

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for olopatadine and olopatadine related compound B are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 2.0 between olopatadine and olopatadine related compound B

Δ USP40

Relative standard deviation: NMT 2.0%,
olopatadine peak

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Olopatadine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity

from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

F = relative response factor for each individual impurity (see Table 1)

Acceptance criteria

Individual impurities: See Table 1. Disregard any peaks corresponding to those of the *Blank solution*,

and any peak less than 0.05%. Δ USP40

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
α -Hydroxy olopatadine ^a	0.4	1.0	0.2
Olopatadine <i>E</i> -isomer ^b	0.7	1.3	0.1
Olopatadine	1.0	—	—
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	0.25

^a Δ (Z)-2-[(11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenzo[*b,e*]oxepin-2-yl)-2-hydroxyacetic acid. Δ USP40

^b Δ 11-[(*E*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenzo[*b,e*]oxepin-2-acetic acid. Δ USP40

SPECIFIC TESTS

• pH (791)

Sample: 10 mg/mL of Olopatadine Hydrochloride in water

Acceptance criteria: 2.0–4.0

• LOSS ON DRYING (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers and store at room temperature.

• USP REFERENCE STANDARDS (11)

USP Olopatadine Hydrochloride RS

USP Olopatadine Related Compound B RS

(Z)-3-{2-[(Carboxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene]-*N,N*-dimethylpropan-1-amine oxide.

$C_{21}H_{23}NO_4$ 353.41

Olopatadine Hydrochloride Ophthalmic Solution

DEFINITION

Olopatadine Hydrochloride Ophthalmic Solution is a sterile aqueous solution of Olopatadine Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of olopatadine ($C_{21}H_{23}NO_3$). It may contain suitable antimicrobial agents.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

Add the following:

- **B.** The UV spectrum in the range of 270–370 nm of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. Δ USP40

ASSAY

Change to read:

• PROCEDURE

Protect all solutions containing olopatadine hydrochloride from light.

Buffer: Dissolve 13.6 g of monobasic potassium phosphate in 1 L of water, add 1 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and Buffer (28:72)

Standard solution: 0.1 mg/mL of USP Olopatadine Hydrochloride RS in *Mobile phase*

Sample solution: Nominally equivalent to 0.1 mg/mL of olopatadine in *Mobile phase*, from Ophthalmic Solution

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 299 nm Δ or diode array. [NOTE—Use a diode array detector to perform the *Identification B* test.] Δ USP40

Column: 4.6-mm \times 15-cm; 5- μ m packing L7

Flow rate: 1 mL/min

Injection volume: 30 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Δ USP40

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of olopatadine ($C_{21}H_{23}NO_3$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Olopatadine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of olopatadine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of olopatadine, 337.41

M_{r2} = molecular weight of olopatadine hydrochloride, 373.87

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

• LIMIT OF EARLY ELUTING IMPURITIES

Protect all solutions containing olopatadine hydrochloride from light.

Mobile phase: Prepare as directed in the Assay.

System suitability solution: 0.2 mg/mL of USP

Olopatadine Hydrochloride RS and 0.02 mg/mL of USP Olopatadine Related Compound B RS in *Mobile phase*

Standard solution: 0.2 mg/mL of USP Olopatadine Hydrochloride RS in *Mobile phase*

Sample solution: Equivalent to 0.2 mg/mL of olopatadine in *Mobile phase*, from Ophthalmic Solution

Blank solution: *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 299 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Flow rate: 1 mL/min

Injection volume: 30 μL

Run time: At least 1.6 times the retention time of the major peak

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between olopatadine and olopatadine related compound B, *System suitability solution*

▲^{USP40}

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of olopatadine from the *Standard solution*

C_S = concentration of USP Olopatadine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of olopatadine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of olopatadine, 337.41

M_{r2} = molecular weight of olopatadine hydrochloride, 373.87

F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peaks corresponding to those of the *Blank solution* and any peaks with a relative retention time, measured with respect to olopatadine, greater than 1.5. ▲Disregard any peak less than 0.1%. ▲^{USP40}

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olopatadine <i>E</i> -isomer ^a	0.7	1.3	0.5
Olopatadine	1.0	—	—
Olopatadine related compound B	1.2	1.0	2
Olopatadine carbaldehyde ^b	1.3	4.5	0.5
Any individual unspecified impurity	—	1.0	0.5

^a ▲11-[(*E*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenzo[*b,e*]oxepin-2-acetic acid. ▲^{USP40}

^b (Z)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenzo[*b,e*]oxepine-2-carbaldehyde.

Change to read:

• **LIMIT OF LATE ELUTING IMPURITIES**

Protect all solutions containing olopatadine hydrochloride from light.

Buffer: Prepare as directed in the Assay.

Mobile phase: Acetonitrile and *Buffer* (1:1)

System suitability solution: 0.02 mg/mL of USP

Olopatadine Hydrochloride RS and 0.01 mg/mL of USP Olopatadine Related Compound C RS in *Mobile phase*

Standard solution: 0.01 mg/mL of USP Olopatadine Related Compound C RS in *Mobile phase*

Sample solution: Use the *Sample solution* from the test for *Limit of Early Eluting Impurities*.

Blank solution: *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 299 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Flow rate: 1 mL/min

Injection volume: 30 μL

Run time: At least 3 times the retention time of the olopatadine related compound C peak

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for olopatadine and olopatadine related compound C are 0.3 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 7.0 between olopatadine and olopatadine related compound C, *System suitability solution*

▲^{USP40}

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of olopatadine related compound C from the *Standard solution*

C_S = concentration of USP Olopatadine Related Compound C RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of olopatadine in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any peaks corresponding to those of the *Blank solution* and any peaks with a relative retention time, measured with respect to olopatadine related compound C, less than 0.7. ▲Disregard any peak less than 0.1%. ▲^{USP40}

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Olopatadine	0.3	—
Olopatadine related compound C	1.0	1
Any individual unspecified impurity	—	0.5
Total impurities ^a	—	3

^a Total impurities are the sum of olopatadine related compound B, olopatadine related compound C, olopatadine *E*-isomer, olopatadine carbaldehyde, and all unspecified impurities found in the tests for *Limit of Early Eluting Impurities* and *Limit of Late Eluting Impurities*.

SPECIFIC TESTS

• **STERILITY TESTS** (71): Meets the requirements

• **PH** (791): 5.0–8.0

• **OSMOLALITY AND OSMOLARITY** (785): 260–340 mOsmol/kg

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 4° and 25°.
- **USP REFERENCE STANDARDS (11)**
 - USP Olopatadine Hydrochloride RS
 - USP Olopatadine Hydrochloride Related Compound B RS (Z)-3-[2-(Carboxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene]-*N,N*-dimethylpropan-1-amine oxide.
C₂₁H₂₃NO₄ 353.41
 - USP Olopatadine Hydrochloride Related Compound C RS 11-Oxo-6,11-dihydrodibenzo[*b,e*]oxepin-2-yl acetic acid.
C₁₆H₁₂O₄ 268.26

Omega-3-Acid Ethyl Esters**DEFINITION**

Omega-3-Acid Ethyl Esters is a mixture of ethyl esters, principally the ethyl esters of eicosapentaenoic acid (EPAee) (C20:5 n-3, EE) and docosahexaenoic acid (DHAee) (C22:6 n-3, EE). It may also contain ethyl esters of alpha-linolenic acid (C18:3 n-3, EE), moroctic acid (C18:4 n-3, EE), eicosatetraenoic acid (C20:4 n-3, EE), heneicosapentaenoic acid (C21:5 n-3, EE), and docosapentaenoic acid (C22:5 n-3, EE). Tocopherol may be added as an antioxidant.

IDENTIFICATION

- **A.** The retention times of the principal peaks in *Test solution 4* correspond to those of eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester in *Standard solution 1b* and *Standard solution 1a*, as obtained in the Assay.
- **B.** It meets the acceptance criteria in *Table 1* of the Assay.

ASSAY

- **CONTENT OF EPAee, DHAee, AND TOTAL OMEGA-3-ACID ETHYL ESTERS**

(See *Fats and Fixed Oils* (401), *Omega-3 Fatty Acids Determination and Profile*.)

Standard solution 1a, Standard solution 1b, Test solution 3, Test solution 4, System suitability solution 1, Chromatographic system, and System suitability: Proceed as directed in *Fats and Fixed Oils* (401), *Omega-3 Fatty Acids Determination and Profile*.

Analysis

Samples: *Standard solution 1a, Standard solution 1b, Test solution 3, and Test solution 4*

Calculate the content of EPAee and DHAee in the portion of Omega-3-Acid Ethyl Esters taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak area ratio of the EPAee or DHAee peak to the internal standard peak from *Test solution 3*

R_S = peak area ratio of the EPAee peak to the internal standard peak from *Standard solution 1b* or DHAee peak to the internal standard peak from *Standard solution 1a*

C_S = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS in *Standard solution 1b* or USP Docosahexaenoic Acid Ethyl Ester RS in *Standard solution 1a* (mg/mL)

C_U = concentration of Omega-3-Acid Ethyl Esters in *Test solution 3* (g/mL)

Calculate the content of total omega-3-acid ethyl esters in the portion of Omega-3-Acid Ethyl Esters taken:

$$\text{Result} = r_{\text{FAn-3ee}} [(EPAee + DHAee)/(r_{EPAee} + r_{DHAee})] + EPAee + DHAee$$

$r_{\text{FAn-3ee}}$ = sum of the peak areas of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), moroctic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), and docosapentaenoic acid ethyl ester (C22:5 n-3, EE) in *Test solution 4*

EPAee = content of EPAee (mg/g)

DHAee = content of DHAee (mg/g)

r_{EPAee} = peak area of EPAee in *Test solution 4*

r_{DHAee} = peak area of DHAee in *Test solution 4*

Acceptance criteria: It conforms to the acceptance criteria in *Table 1*. Articles labeled as Omega-3-Acid Ethyl Esters type A meet *Acceptance Criteria II*.

Table 1

Name	Relative Retention Time	Acceptance Criteria I		Acceptance Criteria II (For articles labeled as Omega-3-Acid Ethyl Esters type A)	
		NLT	NMT	NLT	NMT
C18:3 n-3, EE ^a	0.585	—	—	—	—
C18:4 n-3, EE ^b	0.608	—	—	—	—
C20:4 n-3, EE ^c	0.777	—	—	—	—
C20:5 n-3, EE (EPAee) ^d	0.796	430 mg/g	495 mg/g	365 mg/g	435 mg/g
C21:5 n-3, EE ^e	0.889	—	—	—	—
C22:5 n-3, EE ^f	0.977	—	—	—	—
C22:6 n-3, EE (DHAee) ^g	1.000	347 mg/g	403 mg/g	290 mg/g	360 mg/g
EPAee + DHAee	—	800 mg/g	880 mg/g	700 mg/g	749 mg/g
Total omega-3-acid ethyl esters	—	90% (w/w)	—	78% (w/w)	—

^a Alpha-linolenic acid ethyl ester.

^b Moroctic acid ethyl ester.

^c Eicosatetraenoic acid ethyl ester.

^d Eicosapentaenoic acid ethyl ester.

^e Heneicosapentaenoic acid ethyl ester.

^f Docosapentaenoic acid ethyl ester (clupanodonic acid ethyl ester).

^g Docosahexaenoic acid ethyl ester.

IMPURITIES

- **FATS AND FIXED OILS (401):** NMT 0.1 ppm each of lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg)

• **CHOLESTEROL**

Internal standard stock solution: 3 mg/mL of 5 α -cholestane in *n*-heptane. [NOTE—Prepare fresh before use.]

Internal standard solution: 0.3 mg/mL of 5 α -cholestane in *n*-heptane. [NOTE—Prepare fresh before use.]

Standard stock solution: 3.0 mg/mL of cholesterol in *n*-heptane. [NOTE—This solution is stable for 6 months stored in a freezer.] Transfer 1.0 mL of this solution to a 10.0-mL volumetric flask. Dilute with *n*-heptane to volume. [NOTE—Prepare this solution fresh daily.]

Standard solution: Transfer 1.0 mL each of the *Standard stock solution* and the *Internal standard solution* to a 15-mL centrifuge tube. Prepare as directed in the *Sample solution* beginning with "Evaporate to dryness".

Alpha tocopherol stock solution: 1.5–2.0 mg/mL of USP Alpha Tocopherol RS in *n*-heptane. [NOTE—This solution is stable for 12 months stored in a freezer.]

System suitability solution: Mix 1.0 mL of the *Standard stock solution*, 1.0 mL of the *Internal standard stock solution*, and 2.0 mL of the *Alpha tocopherol stock solution* in a 50-mL volumetric flask. Evaporate to dryness with the aid of heat, and dilute with ethyl acetate to volume. Dilute 1.0 mL of this solution with ethyl acetate to 10.0 mL. [NOTE—This solution is stable for 6 months stored in a freezer.]

Sample solution: Transfer 100 mg of Omega-3-Acid Ethyl Esters to a 15-mL centrifuge tube. Add 1.0 mL of the *Internal standard solution*. Evaporate to dryness at about 50° with a gentle stream of nitrogen. Add 0.5 mL of 50% potassium hydroxide and 3 mL of alcohol, fill the tube with nitrogen, and cap. Heat the sample at 100° for 60 min, using a heating block. Cool for about 10 min. Add 6 mL of water to the tube, and shake for 1 min. Extract the solution four times with 2.5-mL portions of ethyl ether, using a vortex mixer or suitable shaker for 1 min for each extraction. Transfer and combine the extracts into a large centrifuge tube, and wash with 5 mL of water, mixing completely with gentle inversion. Remove the water phase, and add 5 mL of 0.5 M potassium hydroxide to the ether phase, mixing carefully to avoid an emulsion. Remove the potassium hydroxide, and add another 5 mL of water, mixing carefully. Transfer the ether phase to a small centrifuge tube. [NOTE—If an emulsion has occurred, a small amount of sodium chloride may be added to obtain a separation of the phases.] Evaporate the ether phase to dryness under a stream of nitrogen with careful heating. Dissolve the sample in 600 µL of ethyl acetate, and mix well. Transfer 200 µL of this solution to a sample vial, and dilute with ethyl acetate to about 2 mL.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m capillary; coated with a G27 phase of 0.25-µm thickness

Temperatures

Injection port: 320°

Detector: 300°

Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	0	170	1
170	4	320	1.5

Carrier gas: Helium

Flow rate: 1.3 mL/min

Injection volume: 1 µL

Injection type: Splitless injection system

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.2 between alpha tocopherol and cholesterol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the content of total cholesterol in the portion of Omega-3-Acid Ethyl Esters taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U)$$

R_U = peak area ratio of the cholesterol peak to the internal standard from the *Sample solution*

R_S = peak area ratio of the cholesterol peak to the internal standard from the *Standard solution*

W_S = weight of cholesterol in the *Standard solution* (mg)

W_U = weight of Omega-3-Acid Ethyl Esters in the *Sample solution* (g)

Acceptance criteria: NMT 3.0 mg/g

• OLIGOMERS

Mobile phase: Tetrahydrofuran

System suitability solution: Monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin in *Mobile phase*, with concentrations of about 0.5, 0.3, and 0.2 mg/mL, respectively. [NOTE—Suitable grades of monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin may be obtained from Nu-Chek Prep.]

Sample solution 1: 5.0 mg/mL of Omega-3-Acid Ethyl Esters in tetrahydrofuran

Sample solution 2: [NOTE—Use *Sample solution 2* where the results of this test using *Sample solution 1* exceed the *Acceptance criteria* due to the presence of monoglycerides.] Weigh 50 mg of Omega-3-Acid Ethyl Esters into a quartz tube, add 1.5 mL of a 20-g/L solution of sodium hydroxide in methanol, cover with nitrogen, cap tightly with a polytef-lined cap, mix, and heat on a water bath for 7 min. Allow to cool. Add 2.0 mL of boron trichloride-methanol solution, cover with nitrogen, cap tightly, mix, and heat on a water bath for 30 min. Cool to 40°–50°, add 1 mL of isooctane, cap, and shake vigorously for NLT 30 s. Immediately add 5 mL of saturated sodium chloride solution, cover with nitrogen, cap, and shake thoroughly for NLT 15 s. Transfer the upper layer to a separate tube. Shake the methanol layer with 1 mL of isooctane. Wash the combined isooctane extracts with two quantities, each of 1 mL of water. Carefully evaporate the solvent under a stream of nitrogen, then add 10.0 mL of tetrahydrofuran to the residue. Add a small amount of anhydrous sodium sulfate, and filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Differential refractometer

Columns: Three concatenated, 7.8-mm × 30-cm; 7-µm packing L21, with pore sizes in the range of 5–50 nm, arranged with decreasing pore size from the injector to the detector to fulfill the system suitability requirements

Flow rate: 0.8 mL/min

Injection volume: 40 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Elution order: Tridocosahexaenoin, didocosahexaenoin, and monodocosahexaenoin

Resolution: NLT 2.0 between monodocosahexaenoin and didocosahexaenoin; NLT 1.0 between didocosahexaenoin and tridocosahexaenoin

Analysis

Samples: *Sample solution 1* and *Sample solution 2*

Measure the areas of the major peaks.

Calculate the percentage of oligomers in the portion of Omega-3-Acid Ethyl Esters taken to prepare *Sample solution 1*:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = sum of the areas of the peaks with a retention time less than that of the ethyl esters peaks

r_T = sum of the areas of all peaks

Calculate the percentage of oligomers in the portion of Omega-3-Acid Ethyl Esters taken to prepare *Sample solution 2*:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = sum of the areas of all peaks with a retention time less than that of the methyl esters peaks
 r_T = sum of the areas of all peaks

Acceptance criteria: NMT 1.0% of oligomers

- **LIMIT OF DIOXINS, FURANS, AND POLYCHLORINATED BIPHENYLS (PCBs):** Determine the content of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by method No. 1613 revision B of the Environmental Protection Agency. Determine the content of polychlorinated biphenyls (PCBs) by method No. 1668 revision A of the Environmental Protection Agency.

Acceptance criteria: The sum of PCDDs and PCDFs is NMT 1 pg/g of WHO toxic equivalents. The sum of PCBs (polychlorinated biphenyls, IUPAC congeners PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and PCB-180) is NMT 0.5 ppm.

LIMIT OF TOTAL UNIDENTIFIED FATTY ACID ETHYL ESTERS

[NOTE—This test is not required for the articles labeled as Omega-3-Acid Ethyl Esters type A.]

From the chromatogram obtained with *Test solution 4* in the *Assay for Content of EPAee, DHAee, and Total Omega-3-Acid Ethyl Esters*, determine the peak area of the largest single unidentified peak with a relative retention time different from those in *Table 3*.

Table 3

Identified Ethyl Ester	Relative Retention Time
Phytanic acid	0.416
C16:3 n-4	0.431
C16:4 n-1	0.468
C18:3 n-6	0.557
C18:3 n-4	0.574
C18:3 n-3	0.585
C18:4 n-3	0.608
C18:4 n-1	0.618
Furan acid 5	0.691
C19:5	0.710
C20:3 n-6	0.720
C20:4 n-6	0.736
Furan acid 7	0.744
C20:4 n-3	0.777
Furan acid 8	0.783
EPA	0.796
Furan acid 9	0.867
C21:5 n-3	0.889
C22:4	0.917
Furan acid 10	0.922
C22:5 n-6	0.939
Furan acid 11	0.963
C22:5 n-3	0.977
DHA	1.000

Calculate the content of unidentified fatty acid ethyl esters in area percentage:

$$\text{Result} = 100 - (100 \times \Sigma A_{iee}/r_T)$$

A_{iee} = peak area of each identified ethyl ester in *Table 3*

r_T = sum of the areas of all peaks except solvents and BHT

Acceptance criteria: The area of the largest single unidentified peak is NMT 0.5% of the total area. The total area of unidentified peaks as calculated above is NMT 2%.

• **LIMIT OF NON-OMEGA-3-ACID ETHYL ESTERS**

[NOTE—This test is only required for the articles labeled as Omega-3-Acid Ethyl Esters type A.]

From the chromatogram obtained with *Test solution 4* in the *Assay for Content of EPAee, DHAee, and Total Omega-3-Acid Ethyl Esters*, calculate the amounts of C18:1 n-9 ethyl ester and C20:4 n-6 ethyl ester in the portion of Omega-3-Acid Ethyl Esters taken:

$$\text{Result} = (A_{iee}/r_T) \times 100$$

A_{iee} = peak area of C18:1 n-9 ethyl ester or C20:4 n-6 ethyl ester

r_T = sum of the areas of all peaks except solvents and BHT

Acceptance criteria

C18:1 n-9 ethyl ester: NMT 6.0%

C20:4 n-6 ethyl ester: NMT 4.0%

SPECIFIC TESTS

- **FATS AND FIXED OILS (401), Acid Value:** NMT 2.0
- **FATS AND FIXED OILS (401), Anisidine Value:** NMT 15
- **FATS AND FIXED OILS (401), Peroxide Value:** NMT 10.0
- **ABSORBANCE**

Sample solution: Transfer 300 mg, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute immediately with isooctane to volume. Pipet 2.0 mL into a 50-mL volumetric flask, and dilute with isooctane to volume.

Acceptance criteria: NMT 0.55, determined at 233 nm, with isooctane being used as the blank

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers under a nitrogen atmosphere. Store at controlled room temperature.
- **LABELING:** The label states the content of DHA ethyl ester and EPA ethyl ester in mg/g, the sum of the EPA and DHA ethyl esters contents in mg/g, and the content of the total omega-3-acid ethyl esters in weight percentage (w/w). It also states the name of any added antioxidant. Articles intended to meet *Acceptance Criteria II* of the *Assay* and the *Limit of Non-Omega-3-Acid Ethyl Esters* are labeled as Omega-3-Acid Ethyl Esters type A.
- **USP REFERENCE STANDARDS (11)**
 - USP Docosahexaenoic Acid Ethyl Ester RS
All *cis*-4,7,10,13,16,19-docosahexaenoic ethyl ester.
 $C_{24}H_{36}O_2$ 356.55
 - USP Eicosapentaenoic Acid Ethyl Ester RS
All *cis*-5,8,11,14,17-eicosapentaenoic ethyl ester.
 $C_{22}H_{34}O_2$ 330.51
 - USP Methyl Tricosanoate RS
Tricosanoic acid methyl ester.
 $C_{24}H_{48}O_2$ 368.64
 - USP Alpha Tocopherol RS

Omega-3-Acid Ethyl Esters Capsules

DEFINITION

Omega-3-Acid Ethyl Esters Capsules contain Omega-3-Acid Ethyl Esters, with NLT 95.0% and NMT 105.0% of the labeled sum of eicosapentaenoic acid ethyl ester (EPAee) and docosahexaenoic acid ethyl ester (DHAee) and NLT 95% of the labeled amount of total omega-3-acid ethyl esters, as the sum of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), moroctic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), eicosapentaenoic acid ethyl ester (EPAee) (C20:5 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), docosapentaenoic acid ethyl ester (C22:5 n-3, EE), and docosahexaenoic acid ethyl ester (DHAee) (C22:6 n-3, EE). Tocopherol may be added as an antioxidant.

IDENTIFICATION

- **A.** The retention times of the peaks for eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay for Content of EPAee, DHAee, and Total Omega-3-Acid Ethyl Esters.
- **B.** It complies with the Acceptance criteria in the test for Concentration of Omega-3-Acid Ethyl Esters in *Specific Tests*.

ASSAY

• **CONTENT OF EPAee, DHAee, AND TOTAL OMEGA-3-ACID ETHYL ESTERS**

[NOTE—Carry out the procedure as rapidly as possible, avoiding exposure to actinic light, oxidizing agents, oxidation catalysts (i.e., copper and iron), and air.]

Antioxidant solution: 50 mg/L of butylated hydroxytoluene in isooctane

Retention time identification solution: Prepare a mixture containing suitable concentrations of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), moroctic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), and docosapentaenoic acid ethyl ester (C22:5 n-3, EE) in *Antioxidant solution*.¹

Internal standard solution: 7.0 mg/mL of USP Methyl Tricosanoate RS in *Antioxidant solution*

System suitability solution: 5.5 mg/mL of docosahexaenoic acid methyl ester and 0.5 mg/mL of tetracos-15-enoic acid methyl ester in *Antioxidant solution*

Standard solution: Dissolve 60.0 mg of USP Docosahexaenoic Acid Ethyl Ester RS and 90.0 mg of USP Eicosapentaenoic Acid Ethyl Ester RS in 10.0 mL of *Internal standard solution*.

Sample solution: Weigh NLT 10 Capsules in a tared weighing bottle. With a sharp blade, carefully open the Capsules, without loss of shell material, and transfer the combined Capsule contents to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of diethyl ether. Discard the washings, and allow the empty Capsules to air-dry over a period of NMT 30 min, taking precautions to avoid uptake or loss of moisture. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average fill weight per Capsule (W_{AF}). Transfer an amount of the combined Capsule contents equivalent to 225 mg of the labeled amount of total omega-3-acid ethyl esters to a suitable flask, and dissolve with 10.0 mL of *Internal standard solution*.

Chromatographic system

(See *Chromatography* {621}, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 25–50-m fused silica capillary; coated with a 0.25-μm film of G16

Temperatures

Injection port: 250°

Detector: 270°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	0	170	2
170	3.5	255	9

Carrier gas: Hydrogen or helium

Linear velocity: Adjust to obtain a retention time for docosahexaenoic acid ethyl ester of 26 ± 3 min.

¹ The relevant fatty acid ethyl esters are available from Nu-Chek Prep, Inc. (www.nu-chekprep.com); Cayman Chemical (www.caymanchem.com); and Carbosynth (www.carbosynth.com).

Injection volume: 1 μL

Injection type: Split; split ratio, 1:220

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between docosahexaenoic acid methyl ester and tetracos-15-enoic acid methyl ester peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% for the ratios of the peak responses of DHAee and EPAee relative to the internal standard, *Standard solution*

Analysis

Samples: *Retention time identification solution*, *Standard solution*, and *Sample solution*

Identify the retention times of the relevant fatty acid ethyl esters by comparing the peaks from the *Sample solution* with those from the *Retention time identification solution*.

Calculate the content, in mg/g, of EPAee and DHAee in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the *Sample solution*

R_S = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the *Standard solution*

C_S = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS or USP Docosahexaenoic Acid Ethyl Ester RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the total omega-3-acid ethyl esters in the *Sample solution* (g/mL)

Calculate the percentage of the labeled sum of EPAee and DHAee in the portion of Capsules taken:

$$\text{Result} = (EPAee + DHAee) \times W_{AF} \times (100/L)$$

$EPAee$ = content of EPAee in the portion of Capsules taken (mg/g)

$DHAee$ = content of DHAee in the portion of Capsules taken (mg/g)

W_{AF} = average fill weight of the Capsules taken (g)

L = sum of the labeled content of EPAee and DHAee (mg/Capsule)

Calculate the percentage of the labeled amount of total omega-3-acid ethyl esters in the portion of Capsules taken:

$$\text{Result} = \{r_{FAn-3ee} \times [(EPAee + DHAee)/(r_{EPAee} + r_{DHAee})] + EPAee + DHAee\} \times W_{AF} \times (100/L)$$

$r_{FAn-3ee}$ = sum of the peak areas of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), moroctic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), and docosapentaenoic acid ethyl ester (C22:5 n-3, EE) from the *Sample solution*

$EPAee$ = content of EPAee (mg/g)

$DHAee$ = content of DHAee (mg/g)

r_{EPAee} = peak area of EPAee from the *Sample solution*

r_{DHAee} = peak area of DHAee from the *Sample solution*

W_{AF} = average fill weight of the Capsules taken (g)

L = label claim of total omega-3-acids ethyl esters (g/Capsule)

Acceptance criteria: 95.0%–105.0% of the labeled sum of EPAee and DHAee and NLT 95.0% of the labeled amount of total omega-3-acid ethyl esters per Capsule

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905), Weight Variation:** Meet the requirements
- **DISINTEGRATION (701)**
 - Medium, tier 1: Water
 - Medium, tier 2: Simulated gastric fluid TS
 - Time: 30 min
 - Analysis: Perform the test with water as *Medium, tier 1*. Repeat the test with simulated gastric fluid TS as *Medium, tier 2*, if the disintegration time is more than 30 min in *Medium, tier 1*.
 - Acceptance criteria: Meet the requirements

IMPURITIES• **OLIGOMERS**

Mobile phase: Tetrahydrofuran

System suitability solution: Monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin in *Mobile phase*, with concentrations of about 0.5, 0.3, and 0.2 mg/mL, respectively. [NOTE—Suitable grades of monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin may be obtained from Nu-Chek Prep.]

Sample solution 1: 5.0 mg/mL of the Capsule contents in tetrahydrofuran

Sample solution 2: [NOTE—Use *Sample solution 1* where the results of this test using *Sample solution 1* exceed the *Acceptance criteria* due to the presence of monoglycerides.] Weigh 50 mg of the Capsule contents into a quartz tube, add 1.5 mL of a 20-g/L solution of sodium hydroxide in methanol, cover with nitrogen, cap tightly with a polytef-lined cap, mix, and heat on a water bath for 7 min. Allow to cool. Add 2.0 mL of boron trichloride-methanol solution, cover with nitrogen, cap tightly, mix, and heat on a water bath for 30 min. Cool to 40°–50°, add 1 mL of isooctane, cap, and shake vigorously for NLT 30 s. Immediately add 5 mL of saturated sodium chloride solution, cover with nitrogen, cap, and shake thoroughly for NLT 15 s. Transfer the upper layer to a separate tube. Shake the methanol layer with 1 mL of isooctane. Wash the combined isooctane extracts with 2 quantities, each of 1 mL of water. Carefully evaporate the solvent under a stream of nitrogen, then add 10.0 mL of tetrahydrofuran to the residue. Add a small amount of anhydrous sodium sulfate, and filter.

Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: Differential refractometer

Columns: Three concatenated, 7.8-mm × 30-cm; 7-μm packing L21, with pore sizes in the range of 5–50 nm, arranged with decreasing pore size from the injector to the detector to fulfill the system suitability requirements

Flow rate: 0.8 mL/min

Injection volume: 40 μL

System suitability

Sample: *System suitability solution*

Suitability requirements

Elution order: Tridocosahexaenoin, didocosahexaenoin, and monodocosahexaenoin

Resolution: NLT 2.0 between monodocosahexaenoin and didocosahexaenoin; NLT 1.0 between didocosahexaenoin and tridocosahexaenoin

Analysis

Samples: *Sample solution 1* and *Sample solution 2*
Measure the areas of the major peaks.

Calculate the percentage of oligomers in the portion of omega-3-acid ethyl esters taken to prepare *Sample solution 1*:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = sum of the peak areas with retention times less than that of the ethyl esters peak

r_T = sum of the areas of all peaks

Calculate the percentage of oligomers in the portion of the Capsules contents taken to prepare *Sample solution 2*:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = sum of the peak areas with retention times less than that of the methyl esters peak

r_T = sum of the areas of all peaks

Acceptance criteria: NMT 2% of oligomers

SPECIFIC TESTS• **CONCENTRATION OF OMEGA-3-ACID ETHYL ESTERS**

Antioxidant solution, Retention time identification solution, Internal standard solution, System suitability solution, Standard solution, Sample solution, Chromatographic system, System suitability, and Analysis: Proceed as directed in the *Assay for Content of EPAee, DHAee, and Total Omega-3-Acid Ethyl Esters*. Calculate the concentration, in mg/g, of EPAee and DHAee in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the *Sample solution*

R_S = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the *Standard solution*

C_S = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS or USP Docosahexaenoic Acid Ethyl Ester RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the total omega-3-acid ethyl esters in the *Sample solution* (g/mL)

Calculate the concentration, in mg/g, of total omega-3-acids ethyl esters in the portion of Capsules taken:

$$\text{Result} = r_{\text{FA}\omega-3\text{ee}} \times [(EPA\text{ee} + DHA\text{ee})/(r_{EPA\text{ee}} + r_{DHA\text{ee}})] + EPA\text{ee} + DHA\text{ee}$$

$r_{\text{FA}\omega-3\text{ee}}$ = sum of the peak areas of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), morotic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), and docosapentaenoic acid ethyl ester (C22:5 n-3, EE) from the *Sample solution*

EPAee = content of EPAee (mg/g)

DHAee = content of DHAee (mg/g)

$r_{EPA\text{ee}}$ = peak area of EPAee from the *Sample solution*

$r_{DHA\text{ee}}$ = peak area of DHAee from the *Sample solution*

Acceptance criteria: It meets the requirements in *Table 2*. Capsules labeled as containing Omega-3-Acid Ethyl Esters type A meet *Acceptance Criteria II*.

Table 2

Name	Acceptance Criteria I		Acceptance Criteria II (For capsules labeled as containing omega- 3-acid ethyl esters type A)	
	NLT	NMT	NLT	NMT
EPAee	430 mg/g	495 mg/g	365 mg/g	435 mg/g
DHAee	347 mg/g	403 mg/g	290 mg/g	360 mg/g
EPAee + DHAee	800 mg/g	880 mg/g	700 mg/g	749 mg/g
Total omega-3- acid ethyl esters ^a	90% (w/ w)	—	78% (w/ w)	—

^a Sum of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), morotic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), eicosapentaenoic acid ethyl ester (EPAee) (C20:5 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), docosapentaenoic acid ethyl ester (C22:5 n-3, EE), and docosahexaenoic acid ethyl ester (DHAee) (C22:6 n-3, EE).

• **FATS AND FIXED OILS** (401), *Acid Value*

Sample solution: Dissolve about 5.0 g of the oil, accurately weighed, in 100 mL of a mixture of equal volumes of alcohol and ether (which has been neutralized to phenolphthalein with 0.1 M potassium hydroxide) contained in a flask.

Acceptance criteria: NMT 2.0

• **FATS AND FIXED OILS** (401), *Anisidine Value:* NMT 25

• **FATS AND FIXED OILS** (401), *Peroxide Value:* NMT 10 mEq/kg

• **ABSORBANCE**

Sample solution: Transfer 300 mg, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute immediately with isooctane to volume. Pipet 2.0 mL into a 50-mL volumetric flask, and dilute with isooctane to volume.

Acceptance criteria: NMT 0.60, determined at 233 nm in a 1-cm cell, with isooctane being used as the blank

• **MICROBIAL ENUMERATION TESTS** (61): NMT 10^3 cfu/g for the total aerobic microbial count, and NMT 10^2 cfu/g for the total combined yeasts and molds count.

• **TESTS FOR SPECIFIED MICROORGANISMS** (62): Meet the requirements for absence of *Escherichia coli* in 1 g and for absence of *Salmonella* species in 10 g

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature. Do not freeze. Protect from light.

• **LABELING:** The label states the amount of docosahexaenoic acid (DHA) ethyl ester and eicosapentaenoic acid (EPA) ethyl ester, and the minimum amount of total content of omega-3-acid ethyl esters in mg/Capsule. Capsules intended to meet *Acceptance Criteria II* of the test for *Concentration of Omega-3-Acid Ethyl Esters* are labeled as containing Omega-3-Acid Ethyl Esters type A. It also states the name and content of any added antioxidant.

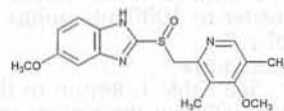
• **USP REFERENCE STANDARDS** (11)

USP Docosahexaenoic Acid Ethyl Ester RS
All *cis*-4,7,10,13,16,19-docosahexaenoic ethyl ester.
 $C_{24}H_{36}O_2$ 356.55

USP Eicosapentaenoic Acid Ethyl Ester RS
All *cis*-5,8,11,14,17-eicosapentaenoic ethyl ester.
 $C_{22}H_{34}O_2$ 330.51

USP Methyl Tricosanoate RS
Tricosanoic acid methyl ester.
 $C_{24}H_{48}O_2$ 368.64

Omeprazole



$C_{17}H_{19}N_3O_3S$ 345.42
1*H*-Benzimidazole, 5-methoxy-2-[[[4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-;
5-Methoxy-2-[[[4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]benzimidazole [73590-58-6].

DEFINITION

Omeprazole contains NLT 98.0% and NMT 102.0% of omeprazole ($C_{17}H_{19}N_3O_3S$), calculated on the dried basis.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. INFRARED ABSORPTION** (197K)

ASSAY

• PROCEDURE

Buffer: Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 1000 mL of water. Dilute 250 mL of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.

Mobile phase: Acetonitrile and *Buffer* (1:3)

Diluent: Acetonitrile and 0.01 M sodium borate (1:3)

Standard solution: 0.2 mg/mL of USP Omeprazole RS in *Diluent*

System suitability solution: 0.1 mg/mL of USP Omeprazole RS in *Diluent* from the *Standard solution*

Sample solution: 0.2 mg/mL of Omeprazole in *Diluent*

Chromatographic system
(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Flow rate: 0.8 mL/min

Injection volume: 20 μL

System suitability

Sample: *System suitability solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of omeprazole ($C_{17}H_{19}N_3O_3S$) in the portion of Omeprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Omeprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm • (Official 1-Jan-2018)

• ORGANIC IMPURITIES

Solution A: Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 1000 mL of water. Dilute 250 mL of this solution with water to 1000 mL. Adjust with phosphoric acid to a pH of 7.0.

Solution B: Acetonitrile

Mobile phase: See Table 1. Return to the original conditions, and re-equilibrate the system for about 10 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
12	75	25
22	50	50
45	50	50
45.1	75	25

Diluent: Solution A and Solution B (3:1)

System suitability solution: 0.6 mg/mL of USP Omeprazole RS, 0.6 µg/mL each of USP Omeprazole Related Compound A RS, USP Omeprazole Related Compound E RS, USP Omeprazole Related Compound I RS, and 5-methoxy-1*H*-benzimidazol-2-thiol in Diluent. [NOTE—This solution is stable for 14 h when stored at 4°.]

Standard solution: 6.0 µg/mL of USP Omeprazole RS in Diluent.

[NOTE—This solution is stable for 14 h when stored at 4°.]

Sample solution: 0.6 mg/mL of Omeprazole in Diluent. [NOTE—Inject within 1 h of preparation.]

Sensitivity solution: 0.3 µg/mL of USP Omeprazole RS from the Standard solution

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 264 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 0.8 mL/min

Injection volume: 40 µL

Autosampler temperature: 4°

System suitability

Samples: System suitability solution, Standard solution, and Sensitivity solution

Suitability requirements

Resolution: NLT 2.0 between omeprazole and omeprazole related compound A, System suitability solution

Relative standard deviation: NMT 5%, Standard solution

Signal-to-noise ratio: NLT 10, Sensitivity solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of any individual impurity in the portion of Omeprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the Sample solution

r_S = peak response of omeprazole from the Standard solution

C_S = concentration of USP Omeprazole RS in the Standard solution (mg/mL)

C_U = concentration of Omeprazole in the Sample solution (mg/mL)

F = relative response factor (see Table 2)

Acceptance criteria: See Table 2. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Methoxy-1 <i>H</i> -benzimidazol-2-thiol	0.41	1.0	0.15
Omeprazole <i>N</i> -oxide (omeprazole related compound E)	0.53	1.34	0.15
Omeprazole sulfone <i>N</i> -oxide (omeprazole related compound I)	0.58	1.48	0.15
Desmethoxy omeprazole ^a	0.97	1.0	0.15
Omeprazole	1.0	—	—
Omeprazole sulfone (omeprazole related compound A)	1.07	1.0	0.15
Omeprazole 4-chloro analog ^b	1.16	1.0	0.15
Ufiprazole ^c	1.25	1.0	0.15
Omeprazole thioxo-pyrido analogs ^d	1.55 and 1.64	—	—
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	1.0

^a 2-[(*RS*)-[(3,5-Dimethylpyridin-2-yl)methyl]sulfinyl]-5-methoxy-1*H*-benzimidazole.

^b 2-[(*RS*)-[4-Chloro-3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-5-methoxy-1*H*-benzimidazole.

^c 5-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylthio]-1*H*-benzimidazole.

^d Omeprazole related compounds F and G (1,3-dimethyl-8-methoxy-12-thioxopyrido[1',2':3,4]imidazo[1,2-*a*]benzimidazol-2(1*2H*)-one and 1,3-dimethyl-9-methoxy-12-thioxopyrido[1',2':3,4]imidazo[1,2-*a*]benzimidazol-2(1*2H*)-one). These impurities are controlled in the test for Limit of Omeprazole Related Compounds F and G.

SPECIFIC TESTS• **COMPLETENESS OF SOLUTION (641)**

Sample solution: 20 mg/mL of Omeprazole in methylene chloride

Acceptance criteria: Meets the requirements

• **LIMIT OF OMEPRAZOLE RELATED COMPOUNDS F AND G**

Sample solution: 20 mg/mL of Omeprazole in methylene chloride

Instrumental conditions

Mode: Vis

Analytical wavelength: 440 nm

Cell: 1 cm

Blank: Methylene chloride

Acceptance criteria: The absorbance is NMT 0.10, corresponding to NMT 350 ppm of the sum of omeprazole related compounds F and G.

• **LOSS ON DRYING (731)**

Analysis: Dry a sample under vacuum at 60° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a cold place, protected from moisture.

• **USP REFERENCE STANDARDS (11)**

USP Omeprazole RS

USP Omeprazole Related Compound A RS

Omeprazole sulfone;

5-Methoxy-2-[[[4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfonyl]-1*H*-benzimidazole.

C₁₇H₁₉N₃O₄S 361.42

USP Omeprazole Related Compound E RS

Omeprazole *N*-oxide;

4-Methoxy-2-[[[*(RS)*-(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl]-3,5-dimethylpyridine 1-oxide.

C₁₇H₁₉N₃O₄S 361.42

USP Omeprazole Related Compound I RS
 Omeprazole sulfone *N*-oxide;
 4-Methoxy-2-[[[(5-methoxy-1*H*-benzimidazol-2-yl)sulfonyl]methyl]-3,5-dimethylpyridine 1-oxide.
 $C_{17}H_{19}N_3O_5S$ 377.41

Omeprazole Delayed-Release Capsules

DEFINITION

Omeprazole Delayed-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_5S$).

IDENTIFICATION

- A.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Solution A: Dissolve 6.0 g of glycine in 1500 mL of water, adjust with 50% sodium hydroxide solution to a pH of 9.0, and dilute with water to 2000 mL.

Solution B: Acetonitrile and methanol (85:15)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	88	12
20	40	60
21	88	12
25	88	12

Diluent: Dissolve 7.6 g of sodium borate decahydrate in about 800 mL of water. Add 1.0 g of edetate disodium, and adjust with 50% sodium hydroxide solution to a pH of 11.0 ± 0.1 . Transfer the solution to a 2000-mL volumetric flask, add 400 mL of dehydrated alcohol, and dilute with water to volume.

Standard solution: 0.2 mg/mL of USP Omeprazole RS in *Diluent*, using sonication as necessary

Sample solution: Weigh and mix the contents of NLT 20 Capsules. Transfer an accurately weighed portion of the Capsule content, equivalent to 20 mg of omeprazole, to a 100-mL volumetric flask, add about 50 mL of *Diluent*, and sonicate for 15 min. Cool, dilute with *Diluent* to volume, mix, and pass through a membrane filter of 0.45- μ m or finer pore size. [NOTE—Bubbles may form just before bringing the solution to volume. Add a few drops of dehydrated alcohol to dissipate the bubbles if they persist for more than a few minutes.]

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 305 nm

Column: 4.6-mm \times 15-cm; 5- μ m base-deactivated packing L7

Flow rate: 1.2 mL/min

Injection size: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 20,000 theoretical plates

Tailing factor: 0.8–2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_5S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of omeprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Test 1

Acid resistance stage

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 100 rpm

Time: 2 h

Buffer C, Mobile phase, Chromatographic system, and System suitability: Proceed as directed for *Buffer stage*.

Standard solution: Transfer 50 mg of USP Omeprazole RS to a 250-mL volumetric flask, dissolve in 50 mL of alcohol, and dilute with 0.01 M sodium borate solution to volume. Transfer 10.0 mL of this solution into a 100-mL volumetric flask, add 20 mL of alcohol, dilute with 0.01 M sodium borate solution to volume, and mix.

Sample solution: After 2 h, filter the *Medium* containing the pellets through a sieve with an aperture of NMT 0.2 mm. Collect the pellets on the sieve, and rinse them with water. Using approximately 60 mL of 0.01 M sodium borate solution, carefully transfer the pellets quantitatively to a 100-mL volumetric flask. Sonicate for about 20 min until the pellets are broken up. Add 20 mL of alcohol to the flask, dilute with 0.01 M sodium borate solution to volume, and mix. Dilute an appropriate amount of this solution with 0.01 M sodium borate solution to obtain a solution containing 0.02 mg/mL. At level L_1 , test 6 units. Test 6 additional units at level L_2 , and at level L_3 , test an additional 12 units. Continue testing through the three levels unless the results conform at either L_1 or L_2 .

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_5S$) dissolved in *Medium*, in mg:

$$\text{Result} = T - C_S \times D \times (r_U/r_S)$$

T = labeled quantity of omeprazole in the capsule (mg)

C_S = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)

D = dilution factor used in preparing the *Sample solution*

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

Tolerances

Level L₁: No individual value exceeds 15% of the omeprazole dissolved.

Level L₂: The average of 12 units is NMT 20% of omeprazole dissolved, and no individual unit is greater than 35% of omeprazole dissolved.

Level L₃: The average of 24 units is NMT 20% of omeprazole dissolved, NMT 2 units are greater than 35% of omeprazole dissolved, and no individual unit is greater than 45% of omeprazole dissolved.

Buffer stage

Medium: pH 6.8 phosphate buffer, 900 mL

Proceed as directed in *Acid resistance stage* with a new set of Capsules from the same batch. After 2 h, add 400 mL of 0.235 M dibasic sodium phosphate to the 500 mL of 0.1 N hydrochloric acid medium in the vessel. Adjust, if necessary, with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8 ± 0.05 .

Apparatus 2: 100 rpm

At the end of 30 min, determine the amount of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved in the pH 6.8 phosphate buffer by using the following method.

Buffer A (0.235 M dibasic phosphate buffer, pH 10.4): 33.36 g/L of anhydrous dibasic sodium phosphate, adjusted with 2 N sodium hydroxide to a pH of 10.4 ± 0.1

Buffer B (phosphate buffer, pH 6.8): 0.1 N hydrochloric acid and *Buffer A* (5:4), adjusted with 2 N hydrochloric acid or 2 N sodium hydroxide, if necessary, to a pH of 6.8 ± 0.05 .

Buffer C (phosphate buffer, pH 7.6): 0.718 g/L of monobasic sodium phosphate and 4.49 g/L of dibasic sodium phosphate, adjusted with 2 N hydrochloric acid or 2 N sodium hydroxide, if necessary, to a pH of 7.6 ± 0.1 . Dilute 250 mL of this solution with water to 1000 mL.

Mobile phase: Transfer 340 mL of acetonitrile to a 1000-mL volumetric flask, dilute with *Buffer C* to volume, and pass through a membrane filter of 0.5- μ m or finer pore size.

Standard solution A (for Capsules labeled to contain 10 mg): Prepare a solution containing 2 mg/mL of USP Omeprazole RS in alcohol. Dilute with *Buffer B* to obtain a solution containing 0.01 mg/mL. Immediately add 2 mL of 0.25 M sodium hydroxide to 10 mL of this solution, and mix. [NOTE—Do not allow the solution to stand before adding the sodium hydroxide solution.]

Standard solution B (for Capsules labeled to contain 20 or 40 mg): Proceed as directed for *Standard solution A*, except to obtain a solution containing 0.02 mg/mL before mixing with 2 mL of 0.25 M sodium hydroxide.

Sample solution A (for Capsules containing 10 or 20 mg): Immediately transfer 5.0 mL of the solution under test to a test tube containing 1.0 mL of 0.25 M sodium hydroxide. Mix well, and pass through a membrane filter of 1.2- μ m or finer pore size. Protect from light.

Sample solution B (for Capsules labeled 40 mg): Immediately transfer 5.0 mL of the solution under test to a test tube containing 2.0 mL of 0.25 M sodium hydroxide and 5 mL of *Buffer B*. Mix well, and pass through a membrane filter of 1.2- μ m or finer pore size. Protect from light.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm \times 12.5-cm; 5- μ m packing L7

Flow rate: 1.0 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution A* or *B*, as appropriate

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved:

$$\text{Result} = (r_u/r_s) \times (C_s/L) \times V \times D \times 100$$

r_u = peak response from the appropriate *Sample solution*

r_s = peak response from the appropriate *Standard solution*

C_s = concentration of the appropriate *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of *Medium*, 900 mL

D = dilution factor used in preparing the appropriate *Sample solution*

Tolerances

For Capsules labeled to contain 10 and 20 mg: NLT 75% (Q) is dissolved.

For Capsules labeled to contain 40 mg: NLT 70% (Q) is dissolved.

The percentages of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved at the time specified conform to *Acceptance Table 1* in *Dissolution* (711).

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Acid resistance stage

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 2 h

Sample solution: After 2 h, remove each sample from the basket, and quantitatively transfer into separate volumetric flasks to obtain a solution having a final concentration of about 0.2 mg/mL. Proceed as directed for the *Sample solution* in the *Assay*, starting with "Add about 50 mL of *Diluent*".

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$) dissolved in *Medium*, in mg:

$$\text{Result} = T - C_s \times D \times (r_u/r_s)$$

T = labeled quantity of omeprazole in the capsule (mg)

C_s = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)

D = dilution factor used in preparing the *Sample solution*

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

Tolerances: See *Table 2*.

Table 2

Level	Criteria
L ₁	The average of the 6 units is NMT 10% of omeprazole dissolved.
L ₂	The average of the 12 units is NMT 10% of omeprazole dissolved.
L ₃	The average of the 24 units is NMT 10% of omeprazole dissolved.

Buffer stage

Medium: 0.05 M pH 6.8 phosphate buffer; 900 mL (see *Reagents, Indicators, and Solutions*)

Apparatus 1: 100 rpm

Time: 45 min

Analysis: Proceed as directed for *Acid resistance stage* with a new set of Capsules from the same batch. After 2 h, replace the acid *Medium* with the buffer *Medium*, and continue the test for 45 more min. Determine the amount of omeprazole (C₁₇H₁₉N₃O₃S) dissolved from UV absorbances at the wavelength of maximum absorbance at about 305 nm on portions of the solutions under test passed through a nylon filter of 0.2-μm pore size, in comparison with a *Standard solution* having a known concentration of USP Omeprazole RS in the same *Medium*.

Tolerances: NLT 75% (Q) is dissolved.

The percentage of the labeled amount of omeprazole (C₁₇H₁₉N₃O₃S) dissolved at the time specified conforms to *Acceptance Table 1* in *Dissolution* (711).

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

IMPURITIES• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 1.0 μg/mL of USP Omeprazole RS in *Diluent*

Peak identification solution: 0.2 mg/mL of USP Omeprazole RS, 1.0 μg/mL of USP Omeprazole Related Compounds F and G Mixture RS, and 1.0 μg/mL of 5-methoxy-1*H*-benzimidazole-2-thiol in *Diluent*. Sonicate the solution for 15 min, and then heat at 55° for 30 min. [NOTE—The heating step facilitates conversion of omeprazole related compounds F and G into a product with the relative retention time of 0.33. The remaining unconverted omeprazole related compounds F and G may elute as a very broad peak at the relative retention time of about 0.5.]

Analysis

Samples: *Standard solution*, *Peak identification solution*, and *Sample solution*

Chromatograph the *Peak identification solution*, and identify the components on the basis of their relative retention times, given in the *Table 3*.

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response for each impurity from the *Sample solution*

r_s = peak response for omeprazole from the *Standard solution*

C_s = concentration of USP Omeprazole RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of omeprazole in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Omeprazole related compounds F and G ^a	0.33	1.6	0.5
5-Methoxy-1 <i>H</i> -benzimidazole-2-thiol	0.64	3.1	0.5
Any other individual impurity	—	1.0	0.5
Total impurities	—	—	2.0

^aThese impurities undergo transformation in the solution to form a conversion product, which elutes at the relative retention time of 0.33.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 15° and 30°.

- **LABELING:** When more than one *Dissolution Test* is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.

• **USP REFERENCE STANDARDS** (11)

USP Omeprazole RS

USP Omeprazole Related Compounds F and G Mixture RS

1,3-Dimethyl-8-methoxy-12-thioxopyrido[1',2':3,4]imidazo[1,2-*a*]benzimidazol-2(1*H*)-one and 1,3-dimethyl-9-methoxy-12-thioxopyrido[1',2':3,4]imidazo[1,2-*a*]benzimidazol-2(1*H*)-one.

Omeprazole Oral Suspension**DEFINITION**

Omeprazole Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of omeprazole (C₁₇H₁₉N₃O₃S).

Prepare Omeprazole Oral Suspension 2 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Omeprazole and sodium bicarbonate for oral suspension ^a equivalent to	200 mg and 16.8 g
Purified Water, USP, a sufficient quantity to make	100 mL

^aZegerid 20-mg/1680-mg powder for oral suspension, Santarus, San Diego, CA.

Calculate the required quantity of each ingredient for the total amount to be prepared. Empty the required number of packets in a suitable mortar. Add *Purified Water* in small portions, and triturate to make a smooth paste. Add increasing volumes of *Purified Water* to make an omeprazole liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Purified Water* to bring to final volume, and mix well.

ASSAY• **PROCEDURE**

Solution A: 50 mM monobasic sodium phosphate buffer, adjusted to pH 8.5 with dilute sodium hydroxide
Mobile phase: Acetonitrile and *Solution A* (25:75). Filter and degas.

Standard stock solution: 1.0 mg/mL of USP Omeprazole RS in *Mobile phase*

Standard solution: 50 μg/mL prepared from *Standard stock solution* in *Mobile phase*

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.25 mL of Oral Suspension

sion into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 50 µg/mL of omeprazole. Centrifuge.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 302 nm

Column: 3.9-mm × 15-cm; 4-µm packing L1

Column temperature: 35°

Flow rate: 1.0 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for omeprazole is about 7.8 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of omeprazole (C₁₇H₁₉N₃O₃S) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of omeprazole in the *Standard solution* (µg/mL)

C_U = nominal concentration of omeprazole in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

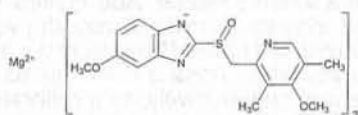
SPECIFIC TESTS

- **PH (791):** 7.5–8.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled cold temperature.
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 45 days after the date on which it was compounded, when stored at controlled cold temperature
- **USP REFERENCE STANDARDS (11)**
USP Omeprazole RS

Omeprazole Magnesium



C₃₄H₃₆MgN₆O₆S₂ 713.12

(*RS*)-5-Methoxy-2-[[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfonyl]-1*H*-benzimidazole, magnesium salt (2:1).

5-Methoxy-2-[[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfonyl]benzimidazole, (*RS*), magnesium salt (2:1) [95382-33-5].

» Omeprazole Magnesium contains not less than 97.5 percent and not more than 102.0 percent of

C₃₄H₃₆MgN₆O₆S₂, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, protected from light. Store at room temperature.

USP Reference standards (11)—

USP Omeprazole RS

USP Omeprazole Magnesium RS

USP Omeprazole Related Compound A RS

Omeprazole sulfone, 5-methoxy-2-[[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfonyl]-1*H*-benzimidazole.

C₁₇H₁₉N₃O₄S 361.42 [CAS-88546-55-8].

Identification—

A: Infrared Absorption (197K).

B: The *Test solution*, prepared and tested as directed in the test for *Content of magnesium*, exhibits a significant absorption at the magnesium emission line at 285.2 nm.

Color of solution—Prepare a solution of Omeprazole Magnesium in methanol having a concentration of 20 mg per mL, and filter. Determine the absorbance of this solution at 440 nm, in 1-cm cells, using methanol as the blank: the absorbance is no greater than 0.1.

Water Determination, Method I (921): between 7% and 10%.

Specific rotation (781S): between +0.5° and −0.5°, measured at 20°.

Test solution: 10 mg per mL, in methanol.

Chromatographic purity—

Phosphate buffer pH 7.6—Prepare as directed in the *Assay*.

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer pH 7.6* and acetonitrile (72.5:27.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—To improve the resolution, the composition may be changed to 75:25, if necessary.]

Test solution—Dissolve about 4 mg of Omeprazole Magnesium in 25 mL of the *Mobile phase*. [NOTE—Prepare this solution fresh.]

System suitability solution—Dissolve about 1 mg of USP Omeprazole RS and 1 mg of USP Omeprazole Related Compound A RS in about 25 mL of *Mobile phase*. [NOTE—Omeprazole related compound A is omeprazole sulfone.]

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 12.5-cm or 4.6-mm × 15-cm column that contains 5-µm packing L7. (Alternatively, a 3.9-mm × 15-cm column that contains 4-µm packing L1 may be used.) The flow rate is about 0.8 to 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times for omeprazole related compound A and omeprazole are about 0.8 and 1.0, respectively; and the resolution, *R*, between omeprazole related compound A and omeprazole is not less than 3.

Procedure—Inject a volume (about 50 µL) of the *Test solution* into the chromatograph, record the chromatogram for at least 4.5 times the retention time of omeprazole, and measure the peak responses. Identify the impurities based on the relative retention times listed in *Table 1*.

Table 1

Name	Relative Retention Time	Limit (%)
Omeprazole <i>N</i> -oxide ¹	0.45	0.1
Omeprazole sulfone ² (Omeprazole related compound A)	0.8	0.1
Omeprazole	1.0	—

¹ 4-Methoxy-2-[[[(*RS*)-(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl]-3,5-dimethylpyridine 1-oxide

² 5-Methoxy-2-[[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfonyl]-1*H*-benzimidazole

Calculate the percentage of any individual impurity in the portion of Omeprazole Magnesium taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response of any individual impurity; and r_s is the sum of the responses of all the peaks: in addition to not exceeding the limits in Table 1, not more than 0.1% of any other individual impurity is found; and not more than 0.5% of total impurities is found.

Content of magnesium—

Lanthanum solution—Transfer 58.7 g of lanthanum oxide to a 1000-mL volumetric flask, wet the substance with some water, and dissolve by cautious addition of 250 mL of hydrochloric acid in 20- to 30-mL portions, cooling between additions. Add water while stirring, cool to room temperature, and dilute with water to volume. [NOTE—Store the solution in a plastic bottle.]

Magnesium standard stock solution—Quantitatively dilute a suitable amount of a commercially prepared atomic absorption standard solution for magnesium with water to obtain a solution containing 1000 µg of magnesium per mL. [NOTE—Store the solution in a plastic bottle.]

Magnesium intermediate standard solution—Transfer 10.0 mL of Magnesium standard stock solution to a 500-mL volumetric flask, add 50 mL of 1 N hydrochloric acid, and dilute with water to volume. Transfer 20.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume. This solution contains 2 µg of magnesium per mL.

Standard solutions—Transfer 5.0, 10.0, 15.0, 20.0, and 25.0 mL of Magnesium intermediate standard solution to separate 100-mL volumetric flasks. To each flask, add 4.0 mL of Lanthanum solution, and dilute with water to volume. These Standard solutions contain 0.1, 0.2, 0.3, 0.4, and 0.5 µg of magnesium per mL, respectively. [NOTE—Concentrations of the Standard solutions and the Test solution may be modified to fit the linear or working range of the instrument. When using instruments with a linear calibration graph, the number of the Standard solutions can be reduced.]

Blank solution—Transfer 4.0 mL of Lanthanum solution to a 100-mL volumetric flask, and dilute with water to volume.

Test solution—Transfer about 250 mg of Omeprazole Magnesium, accurately weighed, to a 100-mL volumetric flask, add 20 mL of 1 N hydrochloric acid, swirl until dissolved, and dilute with water to volume. Allow to stand for 30 minutes. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of the solution so obtained to another 100-mL volumetric flask, add 4.0 mL of Lanthanum solution, and dilute with water to volume.

Procedure—Concomitantly determine the absorbances of the Standard solutions, the Blank solution, and the Test solution at the magnesium emission line at 285.2 nm with a suitable atomic absorption spectrophotometer (see Atomic Absorption Spectroscopy (852)) using an air-acetylene flame. Determine the concentration, C , in µg per mL, of magnesium in the Test solution using the calibration graph. Calculate

the content of magnesium, in percentage, in the portion of Omeprazole Magnesium taken by the formula:

$$100(0.001CD / W)[100 / (100 - L)]$$

in which the multiplier 0.001 is for conversion of µg per mL to mg per mL; C is as defined above; D is the dilution factor for the Test solution; W is the quantity of Omeprazole Magnesium, in mg, taken to prepare the Test solution; and L is the content of water, in percentage, as determined in the test for Water. The magnesium content, calculated on the anhydrous basis, is between 3.30% and 3.55%.

Assay—

Phosphate buffer pH 7.6—Dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, dilute with water to 1000 mL, and mix. Dilute 250 mL of this solution with water to 1000 mL. If necessary, adjust with phosphoric acid to a pH of 7.6.

Mobile phase—Prepare a mixture of Phosphate buffer pH 7.6 and acetonitrile (650:350).

Phosphate buffer pH 11—Mix 11 mL of 0.25 M tribasic sodium phosphate with 22 mL of 0.5 M dibasic sodium phosphate, and dilute with water to 100 mL.

Standard preparation—Transfer about 10 mg of USP Omeprazole RS, accurately weighed, to a 200-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 10 mL of Phosphate buffer pH 11, and dilute with water to volume. This solution contains about 0.05 mg of omeprazole per mL.

Assay preparation—Transfer about 10 mg of Omeprazole Magnesium, accurately weighed, to a 200-mL volumetric flask, dissolve in about 10 mL of methanol, add 10 mL of Phosphate buffer pH 11, and dilute with water to volume. This solution contains about 0.05 mg of omeprazole magnesium per mL.

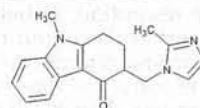
Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 12.5-cm or 4.6-mm × 15-cm column that contains 5-µm packing L7. (Alternatively, a 3.9-mm × 15-cm column that contains 4-µm packing L1 may be used.) The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the column efficiency is not less than 2000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{34}H_{36}MgN_6O_6S_2$ in the portion of Omeprazole Magnesium taken by the formula:

$$100[(713.12 / (2 \times 345.42))(C_s / C_u)(r_u / r_s)]$$

in which 713.12 and 345.42 are the molecular weights of omeprazole magnesium and omeprazole, respectively; C_s is the concentration, in mg per mL, of omeprazole in the Standard preparation; C_u is the concentration, in mg per mL, of omeprazole magnesium in the Assay preparation; and r_u and r_s are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

Ondansetron



$C_{18}H_{19}N_3O$ 293.36

4*H*-Carbazol-4-one, 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]- (±)-
(±)-2,3-Dihydro-9-methyl-3-[(2-methylimidazol-1-yl)methyl]carbazol-4(1*H*)-one [99614-02-5].

» Ondansetron contains not less than 98.0 percent and not more than 102.0 percent of $C_{18}H_{19}N_3O$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers at room temperature.

USP Reference standards (11)—

USP Ondansetron RS

USP Ondansetron Related Compound C RS

1,2,3,9-Tetrahydro-9-methyl-4*H*-carbazol-4-one.

USP Ondansetron Related Compound D RS

1,2,3,9-Tetrahydro-9-methyl-3-methylene-4*H*-carbazol-4-one.

Identification—

A: Infrared Absorption (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Water Determination, Method 1a (921): not more than 3.0%.

Residue on ignition (281): not more than 0.1%.

Chloride (221)—To 1 g of the substance under test, add 30 to 40 mL of water, and warm gently, if necessary, until no more dissolves. Mix well, and pass through a filter paper that gives a negative test for chloride. Add 1 mL of nitric acid and 1 mL of silver nitrate TS. Dilute with water to 50 mL. Mix well, and allow to stand for 5 minutes protected from direct sunlight: any turbidity formed is not greater than that produced in a similarly treated control solution containing 0.3 mL of 0.020 N hydrochloric acid (0.02%).

Limit of ondansetron related compound D—

Phosphate buffer—Dissolve about 2.72 g of monobasic potassium phosphate in 900 mL of water. Adjust with 1 N sodium hydroxide or 0.5 N sodium hydroxide to a pH of 5.4, dilute to 1000 mL, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer* and acetonitrile (80:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard solution—Dissolve an amount of USP Ondansetron Related Compound D RS in *Mobile phase*, and dilute stepwise with *Mobile phase*, to obtain a solution having a known concentration of about 0.4 µg per mL.

Resolution solution—Prepare a solution of USP Ondansetron Related Compound D RS and USP Ondansetron Related Compound C RS in *Mobile phase* having a known concentration of about 0.6 µg per mL and 1.0 µg per mL, respectively.

Test solution—Transfer about 50 mg of Ondansetron, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 328-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The column temperature is maintained at 30°. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for ondansetron related compound C and 1.0 for ondansetron related compound D; and the resolution, *R*, between ondansetron related compound C and ondansetron related compound D is not less than 1.5. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 8000 theoretical plates;

and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of ondansetron related compound D in the ondansetron taken by the formula:

$$10(C/W)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Ondansetron Related Compound D RS in the *Standard solution*; *W* is the weight, in mg, of ondansetron taken to prepare the *Test solution*; and *r_U* and *r_S* are the peak responses of ondansetron related compound D obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.10% is found.

Related compounds—

Phosphate buffer, **Mobile phase**, **Resolution solution**, **Standard preparation**, and **Chromatographic system**—Prepare as directed in the *Assay*.

Test solution—Use the *Assay preparation* prepared as directed in the *Assay*.

Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Ondansetron taken by the formula:

$$100(r_i / r_s)$$

in which *r_i* is the peak area for each impurity; and *r_s* is the sum of the areas of all the peaks: not more than 0.1% of any individual impurity is found; and not more than 0.5% of total impurities is found, including ondansetron related compound D. [NOTE—Disregard the peak corresponding to ondansetron related compound D at a relative retention time of about 0.4.]

Assay—

Phosphate buffer—Dissolve about 2.72 g of monobasic potassium phosphate in 900 mL of water. Adjust with 1 N sodium hydroxide or 0.5 N sodium hydroxide to a pH of 5.4, dilute to 1000 mL, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer* and acetonitrile (52:48). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Resolution solution—Prepare a solution of USP Ondansetron RS and USP Ondansetron Related Compound A RS in *Mobile phase* having a known concentration of about 0.09 mg per mL and 0.05 mg per mL, respectively.

Standard preparation—Dissolve an accurately weighed quantity of USP Ondansetron RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.090 mg per mL.

Assay preparation—Transfer about 45 mg of Ondansetron, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 5.0 mL of this solution into a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 216-nm detector and a 4.6-mm 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 30°. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.1 for ondansetron related compound A and 1.0 for ondansetron; and the resolution, *R*, between ondansetron related compound A and ondansetron is not less than 1.5. Chromatograph the

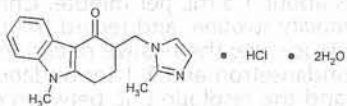
Standard preparation, and record the peak responses as directed for **Procedure**: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 μ L) of the **Standard preparation** and the **Assay preparation** into the chromatograph, record the chromatograms, and measure the responses for the ondansetron peaks. Calculate the quantity, in mg, of $C_{18}H_{19}N_3O$ in the portion of Ondansetron taken by the formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Ondansetron RS in the **Standard preparation**; and r_U and r_S are the peak responses obtained from the **Assay preparation** and the **Standard preparation**, respectively.

Ondansetron Hydrochloride



$C_{18}H_{19}N_3O \cdot HCl \cdot 2H_2O$ 365.85

4*H*-Carbazol-4-one, 1,2,3,9-tetrahydro-9-methyl-3-(2-methyl-1*H*-imidazol-1-yl)methyl-, monohydrochloride, (\pm)-, dihydrate.

(\pm)-2,3-Dihydro-9-methyl-3-(2-methylimidazol-1-yl)methylcarbazol-4(1*H*)-one monohydrochloride dihydrate [103639-04-9].

» Ondansetron Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{18}H_{19}N_3O \cdot HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards (11)—

USP Ondansetron Hydrochloride RS

USP Ondansetron Related Compound A RS

3[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4*H*-carbazol-4-one.

USP Ondansetron Resolution Mixture RS

Ondansetron hydrochloride having approximately 0.4% w/w of both ondansetron related compound A and 6,6'-methylene bis-[(1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)-methyl]-4*H*-carbazol-4-one)].

USP Ondansetron Related Compound C RS

1,2,3,9-Tetrahydro-9-methyl-4*H*-carbazol-4-one.

USP Ondansetron Related Compound D RS

1,2,3,9-Tetrahydro-9-methyl-3-methylene-4*H*-carbazol-4-one.

Identification—

A: **Infrared Absorption** (197M).

B: Dissolve 20 mg in 2 mL of water, add 1 mL of 2 M nitric acid, and filter: the filtrate responds to the test for Chloride (191).

Water Determination, *Method Ia* (921): between 9.0% and 10.5%.

Residue on ignition (281): not more than 0.1%.

Limit of ondansetron related compound D—

Mobile phase—Prepare a filtered and degassed mixture of 0.02 M monobasic potassium phosphate (previously ad-

justed with 1 M sodium hydroxide to a pH of 5.4) and acetonitrile (80:20). Make adjustments if necessary (see **System Suitability** under **Chromatography** (621)).

Standard solution—Dissolve an accurately weighed quantity of USP Ondansetron Related Compound D RS in **Mobile phase**, and dilute quantitatively, and stepwise if necessary, with **Mobile phase** to obtain a solution having a known concentration of about 0.4 μ g per mL.

System suitability solution—Dissolve suitable quantities of USP Ondansetron Related Compound D RS and USP Ondansetron Related Compound C RS in **Mobile phase**, and dilute quantitatively, and stepwise if necessary, with **Mobile phase** to obtain a solution having a concentration of about 0.6 μ g per mL and 1 μ g per mL, respectively.

Test solution—Transfer about 50 mg of Ondansetron Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with **Mobile phase** to volume, and mix.

Chromatographic system (see **Chromatography** (621))—The liquid chromatograph is equipped with a 328-nm detector and a 4.6-mm \times 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the **System suitability solution**, and record the peak responses as directed for **Procedure**: the relative retention times are about 0.8 for ondansetron related compound C and 1.0 for ondansetron related compound D; and the resolution, R , between ondansetron related compound C and ondansetron related compound D is not less than 1.5. Chromatograph the **Standard solution**, and record the peak responses as directed for **Procedure**: the column efficiency determined from the analyte peak is not less than 400 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the **Standard solution** and the **Test solution** into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of ondansetron related compound D in the portion of Ondansetron Hydrochloride taken by the formula:

$$10,000(C/W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Ondansetron Related Compound D RS in the **Standard solution**; W is the weight, in mg, of Ondansetron Hydrochloride taken to prepare the **Test solution**; and r_U and r_S are the peak areas obtained from the **Test solution** and the **Standard solution**, respectively: not more than 0.10% is found.

Chromatographic purity—

METHOD I—

Resolution solution—Dissolve a quantity of USP Ondansetron Resolution Mixture RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of 12.5 mg per mL.

Standard solutions—Dissolve an accurately weighed quantity of USP Ondansetron Hydrochloride RS in methanol, and mix to obtain a solution having a known concentration of about 0.25 mg per mL. Quantitatively dilute this solution with methanol to obtain **Standard solutions**, designated below by letter, having the following compositions:

Standard solution	Dilution	Concentration (μ g RS per mL)	Percentage (% for comparison with test specimen)
A	(1 in 5)	50	0.4
B	(1 in 10)	25	0.2
C	(1 in 20)	12.5	0.1

Test solution—Dissolve an accurately weighed quantity of Ondansetron Hydrochloride in methanol to obtain a solution containing 12.5 mg per mL.

Procedure—Separately apply 20 μ L of the *Test solution*, 20 μ L of each *Standard solution*, and 20 μ L of the *Resolution solution* to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of chloroform, ethyl acetate, methanol, and ammonium hydroxide (90:50:40:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light: complete resolution of the three components of the *Resolution solution* spot is found. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions*: any secondary spot from the chromatogram of the *Test solution* having an R_f value corresponding to that of the uppermost secondary spot of the *Resolution solution* is not larger or more intense than the principal spot obtained from *Standard solution A* (0.4%); and no other secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot obtained from *Standard solution B* (0.2%).

METHOD II—

Mobile phase and Chromatographic system—Proceed as directed in the *Assay*.

Standard solution—Proceed as directed for *Standard preparation* in the *Assay*.

Test solution—Use the *Assay preparation*.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Ondansetron Hydrochloride taken by the formula:

$$50,000(C/W)(1/F)(r_i / r_s)$$

in which C is the concentration, in mg per mL, of USP Ondansetron Hydrochloride RS in the *Standard solution*; W is the weight, in mg, of Ondansetron Hydrochloride taken to prepare the *Test solution*; F is the relative response factor of the impurities as described in the accompanying table; r_i is the peak area for each impurity in the *Test solution*; and r_s is the peak area of ondansetron obtained from the *Standard solution*: it meets the requirements given in the accompanying table.

Compound Name	Relative Retention Time	Relative Response Factor	Limit (%)
Ondansetron related compound C	about 0.32	1.2	0.2
Ondansetron related compound D*	about 0.34	—	0.1
Imidazole	about 0.49	0.3	0.2
2-methylimidazole	about 0.54	0.4	0.2
Ondansetron	1.0	—	—
Ondansetron related compound A	about 1.10	0.8	0.2
Unknown	—	1.0	0.1
Total	—	—	0.5

*Quantified in the test for Limit of ondansetron related compound D.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 0.02 M monobasic sodium phosphate (previously adjusted

with 1 M sodium hydroxide to a pH of 5.4) and acetonitrile (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Ondansetron Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 90 μ g per mL.

System suitability solution—Dissolve suitable quantities of USP Ondansetron Hydrochloride RS and USP Ondansetron Related Compound A RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 90 μ g per mL and 20 μ g per mL, respectively.

Assay preparation—Transfer about 45 mg of Ondansetron Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 5.0 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 216-nm detector and a 4.6-mm \times 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for ondansetron and 1.1 for ondansetron related compound A; and the resolution, R , between ondansetron related compound A and ondansetron is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{19}N_3O \cdot HCl$ in the portion of Ondansetron Hydrochloride taken by the formula:

$$500C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Ondansetron Hydrochloride RS in the *Standard preparation*; and r_u and r_s are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ondansetron Compounded Oral Suspension

DEFINITION

Ondansetron Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$), calculated on the anhydrous basis.

Prepare Ondansetron Compounded Oral Suspension containing 1.0 mg/mL of ondansetron hydrochloride (dihydrate) equivalent to 0.8 mg/mL of ondansetron as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Ondansetron (as ondansetron hydrochloride dihydrate)	80 mg (100 mg)
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

Place the required number of tablets in a suitable glass mortar, and comminute to a fine powder, or add Ondansetron hydrochloride powder. Add 50 mL of the *Vehicle* in 5-mL

portions, and mix well with each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring the preparation to final volume, and mix well.

ASSAY

• PROCEDURE

Mobile phase: 43 mM of monobasic potassium phosphate buffer adjusted with a mixture of 1 N sodium hydroxide and acetonitrile (85:15) to a pH of 5.4

Standard solution: Dissolve USP Ondansetron Hydrochloride RS in *Mobile phase* to obtain a solution with a nominal concentration of 4 µg/mL of ondansetron.

Sample solution: Bring each bottle of Oral Suspension to room temperature. Pipet 500 µL of Oral Suspension from each bottle into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass through a filter of 0.45-µm pore size, and keep frozen at -70° until assayed.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 216 nm

Columns

Guard: 3.9-mm × 20-cm; 4-µm packing L10

Analytical: 4.6-mm × 25-cm; 5-µm packing L10

Flow rate: 1 mL/min

Injection volume: 80 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for ondansetron is about 30 min.]

Suitability requirements

Relative standard deviation: NMT 1.6% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ondansetron (C₁₈H₁₉N₃O) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of ondansetron in the *Standard solution* (µg/mL)

C_U = nominal concentration of ondansetron in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% on the anhydrous basis

SPECIFIC TESTS

- **pH** <791>: 3.6–4.6

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
- **BEYOND-USE DATE:** NMT 42 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator
- **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*. Label to indicate that it contains 0.8 mg/mL of ondansetron equivalent to 1 mg/mL of ondansetron hydrochloride (dihydrate).

• USP REFERENCE STANDARDS <11>

USP Ondansetron Hydrochloride RS

Ondansetron Injection

» Ondansetron Injection is a sterile solution of Ondansetron Hydrochloride in Water for Injection or of Ondansetron in Water for Injection prepared with the aid of Hydrochloric Acid. It may contain suitable buffers and/or tonicity adjusting agents. It contains an amount of Ondansetron Hydrochloride equivalent to not less than 95.0 percent and not more than 105.0 percent of the labeled amount of ondansetron (C₁₈H₁₉N₃O).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, at a temperature between 2° and 30°, protected from light.

USP Reference standards <11>—

USP Endotoxin RS

USP Ondansetron Hydrochloride RS

USP Ondansetron Related Compound A RS

3[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4H-carbazol-4-one.

USP Ondansetron Related Compound C RS

1,2,3,9-Tetrahydro-9-methyl-4H-carbazol-4-one.

USP Ondansetron Related Compound D RS

1,2,3,9-Tetrahydro-9-methyl-3-methylene-4H-carbazol-4-one.

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test <85>—It contains not more than 9.9 USP Endotoxin Units per mg of ondansetron hydrochloride.

pH <791>: between 3.3 and 4.0.

Particulate Matter in Injections <788>: meets the requirements for small-volume injections.

Limit of ondansetron related compound D—

Mobile phase, Standard solution, System suitability solution, and Chromatographic system—Proceed as directed in the test for *Limit of ondansetron related compound D* under *Ondansetron Hydrochloride*.

Test solution—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of ondansetron, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of ondansetron related compound D in the volume of Injection taken by the formula:

$$(2.5 / V)(C_S / C_A)(r_U / r_S)$$

in which V is the volume, in mL, of Injection taken; C_S is the concentration, in µg per mL, of ondansetron related compound D in the *Standard preparation*; C_A is the concentration, in mg per mL, of ondansetron in the Injection, as determined in the *Assay*; and r_U and r_S are the peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 0.12% is found.

Chromatographic purity—

Mobile phase and Chromatographic system—Proceed as directed in the *Assay*.

System suitability solution—Use the *System suitability solution* prepared as directed in the test for *Limit of ondansetron related compound D* under *Ondansetron Hydrochloride*.

Test solution—Use the *Assay preparation*.

Procedure—Inject about 20 μ L of the *System suitability solution*, record the chromatogram, and identify the peaks due to ondansetron related compound C and ondansetron related compound D based on their approximate relative retention times of 0.35 and 0.37, respectively. Inject a volume (about 10 μ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. [NOTE—Ignore the peak due to ondansetron related compound D.] Calculate the percentage of each impurity in the volume of Injection taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all of the peaks: not more than 0.2% of any individual impurity is found, and the total of all impurities, including the percentage of ondansetron related compound D determined in the test for *Limit of ondansetron related compound D*, is not more than 0.5%.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 0.02 M monobasic potassium phosphate (previously adjusted with 1 M sodium hydroxide to a pH of 5.4), and acetonitrile (50:50). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Ondansetron Hydrochloride RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

System suitability solution—Dissolve suitable quantities of USP Ondansetron Hydrochloride RS and USP Ondansetron Related Compound A RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.1 mg per mL and 50 μ g per mL, respectively.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 2 mg of ondansetron, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 216-nm detector and a 4.6-mm \times 20-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for ondansetron and 1.1 for ondansetron related compound A; and the resolution, R , between ondansetron related compound A and ondansetron is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ondansetron ($C_{18}H_{19}N_3O$) in each mL of the Injection taken by the formula:

$$(293.36 / 329.83)(25C / V)(r_U / r_S)$$

in which 293.36 and 329.83 are the molecular weights of ondansetron and anhydrous ondansetron hydrochloride, respectively; C is the concentration, in mg per mL, on the anhydrous basis, of USP Ondansetron Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Injection

taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ondansetron Oral Solution

» Ondansetron Oral Solution is a solution of Ondansetron Hydrochloride in a suitable vehicle. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards (11)—

USP Ondansetron Hydrochloride RS

USP Ondansetron Related Compound A RS

3[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4H-carbazol-4-one.

USP Ondansetron Related Compound C RS

1,2,3,9-Tetrahydro-9-methyl-4H-carbazol-4-one.

USP Ondansetron Related Compound D RS

1,2,3,9-Tetrahydro-9-methyl-3-methylene-4H-carbazol-4-one.

Identification—

A: Thin-Layer Chromatographic Identification Test (201)—

Test solution—Dilute a portion of Oral Solution with a mixture of methanol and water (50:50) to obtain a solution containing about 0.2 mg of ondansetron per mL.

Standard solution: 0.25 mg per mL in methanol.

Developing solvent system: chloroform, ethyl acetate, methanol, and ammonium hydroxide (90:50:40:1).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Escherichia coli*. The total aerobic microbial count does not exceed 100 cfu per g, the *Enterobacteriaceae* count does not exceed 10 cfu per g, and the total combined molds and yeasts count does not exceed 50 cfu per g.

Deliverable volume (698): meets the requirements.

pH (791): between 3.3 and 4.0.

Limit of ondansetron related compound D—

Mobile phase—Proceed as directed in the test for *Limit of ondansetron related compound D* under *Ondansetron Hydrochloride*.

System suitability solution—Dissolve suitable quantities of USP Ondansetron Related Compound D RS and USP Ondansetron Related Compound C RS in *Mobile phase*; and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.5 μ g per mL and 2 μ g per mL, respectively.

Standard solution—Dissolve an accurately weighed quantity of USP Ondansetron Related Compound D RS in *Mobile phase*; and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.5 μ g per mL.

Test solution—Quantitatively dilute, if necessary, an accurately measured volume of Oral Solution with *Mobile phase* to obtain a solution containing about 0.8 mg of ondansetron per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 328-nm detector and a 4.6-mm \times 25-cm column that contains packing L10.

The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between ondansetron related compound D and ondansetron related compound C is not less than 2.0; the tailing factor for ondansetron related compound D is not more than 2.0; and the relative standard deviation for replicate injections is not more than 4.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of ondansetron related compound D in the volume of Oral Solution taken by the formula:

$$100D(C_S / C_A)(r_U / r_S)$$

in which *D* is the dilution factor for the Oral Solution in the *Test solution*; *C_S* is the concentration, in μ g per mL, of USP Ondansetron Related Compound D RS in the *Standard solution*; *C_A* is the concentration, in μ g per mL, of ondansetron in the Oral Solution, as determined in the *Assay*; and *r_U* and *r_S* are the peak responses of ondansetron related compound D obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.1% is found.

Related compounds—

Mobile phase, System suitability solution, and Chromatographic system—Proceed as directed in the *Assay* under *Ondansetron Hydrochloride*.

Standard solution—Prepare as directed for the *Standard preparation*, in the *Assay* under *Ondansetron Hydrochloride*.

Test solution—Use the *Assay preparation*.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each related compound in the volume of Oral Solution taken by the formula:

$$(293.36 / 329.83)10,000(1 / F)(1 / V)(C_S / C_A)(r_i / r_S)$$

in which 293.36 and 329.83 are the molecular weights of ondansetron and anhydrous ondansetron hydrochloride, respectively; *F* is the relative response factor for each known and unknown impurity (the values of relative response factors [RRF] and the limits can be obtained from Table 1); *V* is the volume, in mL, of Oral Solution taken; *C_S* is the concentration, in mg per mL, on the anhydrous basis, of USP Ondansetron Hydrochloride RS in the *Standard solution*; *C_A* is the concentration, in mg per mL, of ondansetron in the Oral Solution; *r_i* is the peak response for any related compound obtained from the *Test solution*; and *r_S* is the peak response for ondansetron obtained from the *Standard solution*.

Table 1

Related Compound	Approx. RRT	RRF	Limit (%)
Ondansetron related compound D*	0.34	—	0.1
Imidazole	0.40	0.46	0.2
2-Methyl imidazole	0.53	0.54	0.2
Des-C-methyl ondansetron hydrochloride	0.62	0.76	0.2
N-Desmethyl ondansetron maleate	0.83	0.73	0.2
Ondansetron related compound A	1.2	0.81	0.2
Unknown		1.0	0.2
Total (including ondansetron related compound D)		—	0.5

*Quantified from Limit of related compound D test

Assay—

Mobile phase, System suitability solution, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay* under *Ondansetron Hydrochloride*.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 9 mg of ondansetron, to a 100-mL volumetric flask; dilute with *Mobile phase* to volume; and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 216-nm detector and a 4.6-mm \times 25-cm column that contains packing L10. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.1 for ondansetron related compound A and 1.0 for ondansetron; and the resolution, *R*, between ondansetron related compound A and ondansetron is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ondansetron ($C_{18}H_{19}N_3O$) in each mL of Oral Solution taken by the formula:

$$(293.36 / 329.83)100(C / V)(r_U / r_S)$$

in which 293.36 and 329.83 are the molecular weights of ondansetron and anhydrous ondansetron hydrochloride, respectively; *C* is the concentration, in mg per mL, on the anhydrous basis, of USP Ondansetron Hydrochloride RS in the *Standard preparation*; *V* is the volume, in mL, of Oral Solution taken; and *r_U* and *r_S* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ondansetron Tablets

DEFINITION

Ondansetron Tablets contain Ondansetron Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ondansetron ($C_{18}H_{19}N_3O$).

IDENTIFICATION

A. INFRARED ABSORPTION (197K)

Sample: Transfer a portion of the powder from finely powdered Tablets, equivalent to 100 mg of ondansetron hydrochloride, to a suitable conical flask. Add 50 mL of alcohol, and swirl. Pass the liquid through a PTFE filter of 0.45- μ m pore size into a 50-mL beaker. Evaporate the solvent on a rotary evaporator. Dry the precipitate in an air oven for 1 h at 105°. Prepare a suitable dispersion of the residue in potassium bromide, and record the spectra of the *Sample* and the standard specimen in the spectral range 3800–650 cm^{-1} . [NOTE—It is recommended that a solution of USP Ondansetron Hydrochloride RS in alcohol be prepared at a concentration of 2 mg/mL before the evaporation, followed by the drying steps.]

Acceptance criteria: The *Sample* shows strong bands at 1621, 1481, 1281, and 758 cm^{-1} , similar to the potassium bromide dispersion of USP Ondansetron Hydrochloride RS.

- B.** The retention time of the major peak of the *Sample* solution corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Buffer: 2.7 g/L of monobasic potassium phosphate. Adjust with 1 N sodium hydroxide to a pH of 5.4.

Mobile phase: Acetonitrile and Buffer (1:4)

Diluent: Acetonitrile and Buffer (1:1)

Standard solution: 0.05 mg/mL of ondansetron (free base) in Diluent from USP Ondansetron Hydrochloride RS

Sample stock solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 50 mg of ondansetron, based on the label claim, to a 100-mL volumetric flask. Add 70 mL of Diluent, and sonicate for about 20 min. Dilute with Diluent to volume. Centrifuge a portion of the solution.

Sample solution: Quantitatively dilute the supernatant with Diluent to obtain a solution having a nominal concentration of 0.05 mg/mL of ondansetron, based on the label claim. Pass through a suitable nylon filter of 0.45-μm pore size, and use the filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 216 nm

Column: 4.6-mm × 25-cm; 5-μm packing L10

Flow rate: 1.5 mL/min

Injection size: 10 μL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the label claim of ondansetron ($C_{18}H_{19}N_3O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of ondansetron (free base) in the Standard solution (mg/mL)

C_U = nominal concentration of ondansetron in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS**• DISSOLUTION (711)****Test 1**

Medium: Water; 500 mL, deaerated

Apparatus 2: 50 rpm

Time: 15 min

Standard solution: USP Ondansetron Hydrochloride RS in Medium in a concentration similar to the one expected in the Sample solution

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size, and dilute, if necessary, with Medium.

Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

Mode: UV

Analytical wavelength: 310 nm

Blank: Medium

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of ondansetron ($C_{18}H_{19}N_3O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

A_U = absorbance of the Sample solution

A_S = absorbance of the Standard solution

C_S = concentration of the Standard solution (mg/mL)

L = label claim (mg/Tablet)

M_{r1} = molecular weight of ondansetron, 293.36

M_{r2} = molecular weight of ondansetron hydrochloride (anhydrous), 329.83

V = volume of Medium, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of $C_{18}H_{19}N_3O$ is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

Medium, Apparatus 2, Standard solution, Sample solution, Instrumental conditions, and Analysis: Proceed as directed for Test 1.

Time: 30 min

Tolerances: NLT 80% (Q) of the labeled amount of $C_{18}H_{19}N_3O$ is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 3.

Medium: 0.01 N hydrochloric acid; 500 mL, deaerated

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: Known concentration of USP Ondansetron Hydrochloride RS in Medium, close to the expected concentration of the Sample solution

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size, and dilute, if necessary, with Medium.

Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

Mode: UV

Analytical wavelength: 248 nm

Blank: Medium

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of ondansetron ($C_{18}H_{19}N_3O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

A_U = absorbance of the Sample solution

A_S = absorbance of the Standard solution

C_S = concentration of the Standard solution (mg/mL)

L = label claim (mg/Tablet)

M_{r1} = molecular weight of ondansetron, 293.36

M_{r2} = molecular weight of ondansetron hydrochloride (anhydrous), 329.83

V = volume of Medium, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of $C_{18}H_{19}N_3O$ is dissolved.

Test 4: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 4.

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard stock solution: 450 μg/mL of USP Ondansetron Hydrochloride RS in Medium

Standard solution: Dilute the Standard stock solution quantitatively and stepwise, if necessary, with Medium to obtain a final concentration of about (L/500) mg/mL, where L is the Tablet label claim, in mg.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

Mode: UV

Analytical wavelength: 249 nm

Cell path: 1 cm for Tablets labeled to contain 4 or 8 mg; 0.2 cm for Tablets labeled to contain 16 or 24 mg

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ondansetron ($C_{18}H_{19}N_3O$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

M_{r1} = molecular weight of ondansetron, 293.36

M_{r2} = molecular weight of ondansetron hydrochloride (anhydrous), 329.83

V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of ondansetron is dissolved.

Test 5: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium, Apparatus 2, Standard solution, Sample solution, Instrumental conditions, and Analysis: Proceed as directed for *Test 1*.

Time: 30 min

Tolerances: NLT 70% (Q) of the labeled amount of ondansetron is dissolved.

Test 6: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium: Water; 500 mL, deaerated

Apparatus 2: 50 rpm

Time: 30 min

Buffer: 3.12 g/L of monobasic sodium phosphate dihydrate. Adjust with 1 N sodium hydroxide to a pH of 5.4.

Mobile phase: Acetonitrile and *Buffer* (40:60)

Standard solution

For Tablets labeled to contain 4 or 24 mg: 0.01 mg/mL of USP Ondansetron Hydrochloride RS in *Medium*

For Tablets labeled to contain 8 mg: 0.02 mg/mL of USP Ondansetron Hydrochloride RS in *Medium*

Sample solution

For Tablets labeled to contain 4 or 8 mg: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

For Tablets labeled to contain 24 mg: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size. Further dilute 4.0 mL of this solution with *Medium* to 25.0 mL.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 216 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L10

Flow rate: 2.0 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of ondansetron ($C_{18}H_{19}N_3O$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times D \times 100$$

r_U = peak response of the *Sample solution*

r_S = peak response of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

M_{r1} = molecular weight of ondansetron, 293.36

M_{r2} = molecular weight of ondansetron hydrochloride (anhydrous), 329.83

V = volume of *Medium*, 500 mL

D = dilution factor of the *Sample solution*

Tolerances: NLT 75% (Q) of the labeled amount of ondansetron is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer and Mobile phase: Proceed as directed in the *Assay*.

System suitability solution: 0.05 and 0.1 mg/mL of USP Ondansetron Related Compound A RS and USP Ondansetron Hydrochloride RS, respectively, in *Mobile phase*

Standard stock solution: Use the *Standard solution* in the *Assay*.

Standard solution: 1.5 μ g/mL of ondansetron in *Mobile phase* from the *Standard stock solution*

Sample solution: Weigh and crush NLT 20 Tablets.

Transfer a quantity of powder, equivalent to 50 mg of ondansetron, to a 100-mL volumetric flask. Add about 70 mL of *Mobile phase*, and sonicate for about 20 min. Dilute with *Mobile phase* to volume. Centrifuge the solution. Pass a portion of the solution through a suitable nylon filter of 0.45- μ m pore size, and use the filtrate.

Chromatographic system: Proceed as directed in the *Assay*.

Run time: At least 45 min for the *Sample solution*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between ondansetron related compound A and ondansetron, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of ondansetron from the *Standard solution*

C_S = concentration of ondansetron (free base) in the *Standard solution* (mg/mL)

C_U = nominal concentration of ondansetron in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Methyl imidazole ^a	0.22	0.53	0.2
Ondansetron related compound C ^b	0.40	1.2	0.2

^a Not to be included in total impurities.

^b 1,2,3,9-Tetrahydro-9-methyl-4H-carbazol-4-one.

^c 1,2,3,9-Tetrahydro-9-methyl-3-methylene-4H-carbazol-4-one.

^d 3[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4H-carbazol-4-one.

^e 1,2,3,9-Tetrahydro-9-methyl-3-[1H-imidazol-1-yl]methyl]-4H-carbazol-4-one.

Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ondansetron related compound D ^c	0.47	1.3	0.1
Ondansetron related compound A ^d	0.87	0.90	0.2
Desmethylandansetron ^{a,e}	0.90	0.91	0.2
Ondansetron	1.0	—	—
Any other individual, unspecified degradation product	—	1.0	0.2
Total impurities	—	—	1.0

^a Not to be included in total impurities.^b 1,2,3,9-Tetrahydro-9-methyl-4H-carbazol-4-one.^c 1,2,3,9-Tetrahydro-9-methyl-3-methylene-4H-carbazol-4-one.^d 3[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4H-carbazol-4-one.^e 1,2,3,9-Tetrahydro-9-methyl-3-[1H-imidazol-1-yl)methyl]-4H-carbazol-4-one.**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
 - USP Ondansetron Hydrochloride RS
 - USP Ondansetron Related Compound A RS
 - 3[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4H-carbazol-4-one.

Ondansetron Orally Disintegrating Tablets

DEFINITION

Ondansetron Orally Disintegrating Tablets contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of ondansetron (C₁₈H₁₉N₃O).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Diluent: 0.01 N hydrochloric acid

Buffer: 2.72 g/L of monobasic potassium phosphate in water. Adjust with 1 N sodium hydroxide or 0.5 N sodium hydroxide to a pH of 5.4.

Mobile phase: Acetonitrile and *Buffer* (48:52)

Standard solution: 40 µg/mL of USP Ondansetron RS in *Diluent*

System suitability solution: 0.02 mg/mL of USP Ondansetron Related Compound A RS and 0.006 mg/mL of USP Ondansetron RS in *Diluent*

Sample stock solution: Equivalent to 400 µg/mL of ondansetron. Transfer 10 Tablets to a suitable volumetric flask. Add *Diluent* to fill about 60% of the flask capacity. Shake by mechanical means for about 5 min, and dilute with *Diluent* to volume. Filter a portion of this solution through a polypropylene membrane of 0.45-µm pore size, discarding the first 5 mL of the filtrate.

Sample solution: 40 µg/mL of ondansetron in *Diluent*, from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 216 nm

Column: 4.6-mm × 25-cm; packing L10

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ondansetron and ondansetron related compound A are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.5 between ondansetron related compound A and ondansetron, *System suitability solution*

Tailing factor: NMT 2.0 for the ondansetron peak, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ondansetron (C₁₈H₁₉N₃O) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ondansetron RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ondansetron in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISINTEGRATION (701):** NMT 10 s

- **DISSOLUTION (711)**

Medium: 0.1 N hydrochloric acid; 500 mL, deaerated

Apparatus 2: 50 rpm

Time: 10 min

Detector: UV 310 nm

Cell: 1 cm for 4-mg and 8-mg Tablets; 0.5 cm for 16-mg Tablets; 0.2 cm for 24-mg Tablets

Standard solution: (L/500) mg/mL of USP Ondansetron RS in *Medium*, where L is the label claim in mg

Sample solution: Pass a portion of the solution under test through a suitable filter.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of ondansetron released:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Ondansetron RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of C₁₈H₁₉N₃O is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES• **ORGANIC IMPURITIES**

Buffer: Prepare as directed in the *Assay*.

Mobile phase: Acetonitrile and *Buffer* (1:4)

Standard solution: 2 µg/mL of USP Ondansetron RS in *Mobile phase*

System suitability solution: 2 µg/mL each of USP Ondansetron Related Compound D RS, 2-methylimidazole, and USP Ondansetron RS in *Mobile phase*. [NOTE—First dissolve in acetonitrile, then dilute with *Mobile phase* to volume.]

System sensitivity solution: 0.2 µg/mL of USP Ondansetron RS, from the *Standard solution* in *Mobile phase*

Sample solution: Equivalent to 400 µg/mL of ondansetron. Transfer 10 Tablets to a suitable volumetric flask. Add *Mobile phase* to fill about 60% of the flask capacity. Shake by mechanical means for about 5 min, and dilute with *Mobile phase* to volume. Centrifuge a portion of this solution at 3000 rpm for 10 min. Use the supernatant.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 216 nm

Column: 4.6-mm × 25-cm; packing L10

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Samples: *Standard solution*, *System suitability solution*, and *System sensitivity solution*

Suitability requirements

Resolution: NLT 1.5 between ondansetron and any adjacent peak, *System suitability solution*

Column efficiency: NLT 8000 theoretical plates for ondansetron, *System suitability solution*

Tailing factor: NMT 2.0 for the ondansetron peak, *System suitability solution*

Signal-to-noise ratio: NLT 15, *System sensitivity solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak area of any impurity in the *Sample solution*

r_s = peak area of ondansetron from the *Standard solution*

C_s = concentration of USP Ondansetron RS in the *Standard solution* (µg/mL)

C_u = nominal concentration of the *Sample solution* (µg/mL)

F = relative response factor for each impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

[NOTE—The run time is about 60 min.]

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Methylimidazole	0.16	0.5	0.2
Ondansetron related compound D	0.45	1.2	0.12
Ondansetron	1.0	—	—
Individual unknown impurity	—	1.0	0.2
Total impurities	—	—	0.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant containers. Store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Ondansetron RS

USP Ondansetron Related Compound A RS

3[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4H-carbazol-4-one.

USP Ondansetron Related Compound D RS

1,2,3,9-Tetrahydro-9-methyl-3-methylene-4H-carbazol-4-one.

Bland Lubricating Ophthalmic Ointment

DEFINITION

Bland Lubricating Ophthalmic Ointment is a sterile ointment of white petrolatum and mineral oil. It may contain Lanolin, Modified Lanolin, or Lanolin Alcohols. It may also contain a suitable antimicrobial preservative.

SPECIFIC TESTS

• APPEARANCE

Analysis: Transfer a portion of it to a suitable test tube, and examine the sample in front of a light source.

Acceptance criteria: The sample appears translucent.

• STERILITY TESTS (71):

Meets the requirements

• ACIDITY OR ALKALINITY

Sample solution: Transfer 20.0 g of Ophthalmic Ointment to a 400-mL beaker. Add 100 mL of a mixture of neutralized alcohol and water (1 in 2), agitate thoroughly, and gradually heat to boiling. Boil for 10 min.

Analysis: To the *Sample solution* add 1 mL of phenolphthalein TS, and rapidly titrate with vigorous agitation with either 0.1 N sodium hydroxide VS (from a colorless alcohol-water layer to a sharp pink endpoint) or 0.1 N hydrochloric acid VS (from a pink alcohol-water layer to a colorless endpoint).

Acceptance criteria: NMT 0.40 mL of 0.1 N hydrochloric acid VS or 0.1 N sodium hydroxide VS is required to produce the color change.

• COLOR

Analysis: Examine the extruded Ophthalmic Ointment for color.

Acceptance criteria: Colorless to light yellow

• OTHER REQUIREMENTS:

It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in suitable collapsible ophthalmic ointment tubes.

Opium

» Opium is the air-dried milky exudate obtained by incising the unripe capsules of *Papaver somniferum* L. or its variety *album* De Candolle (Fam. Papaveraceae). It yields not less than 9.5 percent of anhydrous morphine.

Botanic characteristics—More or less rounded, oval, brick-shaped or elongated, somewhat flattened masses usually about 8 cm to 15 cm in diameter and weighing about 300 g to 2 kg each. Externally, it is pale olive-brown or olive-gray, having a coarse surface and being covered with a thin coating consisting of fragments of poppy leaves and, at times, with fruits of a species of *Rumex* adhering from the packing; it is more or less plastic when fresh, becoming hard or tough on storage. Internally, it is reddish brown and coarsely granular.

Assay—

Chromatographic tubes, Citrate buffer, and Standard preparation—Prepare as directed in the Assay under *Paregoric*.

Assay preparation—Transfer about 2 g of Opium, accurately weighed, to a 250-mL beaker, add 20 mL of dimethyl sulfoxide, and heat for 20 minutes on a steam bath, intermittently dispersing the substance with a flat-end stirring rod. Allow to stand for 15 minutes to permit undissolved material to settle, and carefully decant the supernatant into a 100-mL volumetric flask. Add another 20 mL of dimethyl sulfoxide to the residue, rinsing the sides of the beaker with dimethyl sulfoxide. Disperse and heat the substance as before, allow to settle, and decant into the volumetric flask. Repeat the dissolution one or two times, until the opium is dissolved (other than for small leaf fragments, sand-like particles, gelatinous materials, etc.). Rinse the beaker, and transfer the residue to the flask with the aid of water. Dilute with water to about 90 mL, and mix. If necessary, add 1 drop of alcohol to dispel any foam. Cool to room temperature, adjust with water to volume, and mix. Pass the resulting solution through a medium-porosity filter paper, discarding the first 20 mL of the filtrate.

Chromatographic columns—Pack a pledget of glass wool at the base of each of the three tubes, and fill with adsorbent using chromatographic siliceous earth as the base of the adsorbent, and tamping it firmly in place. Prepare the tubes as follows. Pack *Column I* in two layers, the lower layer consisting of 3 g of chromatographic siliceous earth mixed with 2 mL of *Citrate buffer* and the upper layer consisting of 3 g of chromatographic siliceous earth mixed with 2.0 mL of the *Assay preparation* and 0.5 mL of *Citrate buffer*. Dry-rinse the beaker in which the components of the two layers have been mixed with 1 g of chromatographic siliceous earth, and add it also to the top of *Column I*. Pack *Column II* with 3 g of chromatographic siliceous earth mixed with 2 mL of dibasic potassium phosphate solution (1 in 5.75). Pack *Column III* with 3 g of chromatographic siliceous earth mixed with 2 mL of sodium hydroxide solution (1 in 50). Place a small pad of glass wool above each column packing.

Procedure—Proceed as directed in the Assay under *Paregoric*. Calculate the percentage of anhydrous morphine in the Opium taken by the formula:

$$0.25(C/W)(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of anhydrous morphine in the *Standard preparation*; *W* is the weight, in g, of Opium taken; and *A_U* and *A_S* are the corrected absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Powdered Opium

» Powdered Opium is Opium dried at a temperature not exceeding 70°, and reduced to a very fine powder.

Powdered Opium yields not less than 10.0 percent and not more than 10.5 percent of anhydrous morphine. It may contain any of the diluents, with the exception of starch, permitted for powdered extracts under *Extracts* (1151).

Packaging and storage—Preserve in well-closed containers.

Botanic characteristics—Consists chiefly of yellowish brown to yellow, more or less irregular and granular fragments of latex, varying from 15 to 150 µm in diameter; a

few fragments of strongly lignified, thick-walled, 4- to 5-sided or narrowly elongated, epidermal cells of the poppy capsule; very few fragments of tissues of poppy leaves, poppy capsules, and, occasionally, *Rumex* fruits. In addition, there will be the microscopic characteristics of the diluent if any has been used in the preparation of the powder.

Assay—Proceed with Powdered Opium as directed in the Assay under *Opium*.

Opium Tincture

» Opium Tincture contains, in each 100 mL, not less than 0.90 g and not more than 1.10 g of anhydrous morphine.

Opium Tincture may be prepared as follows.

Place 100 g of granulated or sliced Opium in a suitable vessel. [NOTE—Do not use Powdered Opium.] Add 500 mL of boiling water, and allow to stand, with frequent stirring, for 24 hours. Transfer the mixture to a percolator, allow it to drain, percolate with water as the menstruum to complete extraction, and evaporate the percolate to a volume of 400 mL. Boil actively for not less than 15 minutes, and allow to stand overnight. Heat the mixture to 80°, add 50 g of paraffin, and heat until the paraffin is melted. Beat the mixture thoroughly, and cool.

Remove the paraffin, and filter the concentrate, washing the paraffin and the filter with sufficient water to make the filtrate measure 750 mL. Add 188 mL of alcohol to the filtrate, mix, and assay a 10-mL portion of the resulting solution as directed in the Assay. Dilute the remaining solution with a mixture of 1 volume of alcohol and 4 volumes of water to obtain a Tincture containing 1 g of anhydrous morphine in each 100 mL. Mix.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

Alcohol Determination (611): between 17.0% and 21.0% of C₂H₅OH, determined by the gas-liquid procedure, acetone being used as the internal standard.

Assay—

Chromatographic tubes, Citrate buffer, Standard preparation, and Chromatographic columns—Prepare as directed in the Assay under *Paregoric*.

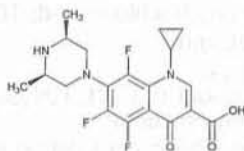
Assay preparation—Transfer 10.0 mL of Tincture to a 50-mL volumetric flask containing 10.0 mL of alcohol, add purified water to volume, and mix. Transfer a 2.0-mL aliquot of the solution, equivalent to about 4 mg of Morphine, to a 50-mL beaker, and add 0.5 mL of *Citrate buffer*.

Procedure—Proceed as directed for *Procedure* in the Assay under *Paregoric*. Calculate the weight of anhydrous morphine, in g per 100 mL of the Tincture taken by the formula:

$$0.250W(A_U / A_S)$$

in which *W* is the weight, in mg, of anhydrous morphine in the 50 mL of *Standard preparation*, and *A_U* and *A_S* are the corrected absorbances of the solution from the *Assay preparation* and the *Standard preparation*, respectively.

Orbifloxacin



$C_{19}H_{20}F_3N_3O_3$ 395.38
1-Cyclopropyl-7-(*cis*-3,5-dimethyl-1-piperazinyl)-5,6,8-trifluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid [113617-63-3].

» Orbifloxacin contains not less than 98.5 percent and not more than 101.5 percent of $C_{19}H_{20}F_3N_3O_3$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers. Store at room temperature.

USP Reference standards (11)—
USP Orbifloxacin RS

Identification—

A: *Infrared Absorption* (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

C: *X-Ray Diffraction* (941)—The X-ray diffraction pattern conforms to that of USP Orbifloxacin RS, similarly determined.

Microbial enumeration tests (61)—The total combined molds and yeasts count does not exceed 100 cfu per g.

pH (791): between 6.5 and 7.8, in a solution containing 10 mg per mL.

Water Determination, Method 1c (921): between 1.5% and 2.9%.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals, Method II** (231): not more than 20 ppm.

• (Official 1-Jan-2018)

Related compounds—

Buffer, Mobile phase, System suitability preparation, Standard preparation, and Chromatographic system—Prepare as directed in the *Assay*.

Standard solution—Dilute, quantitatively with *Buffer*, the *Standard preparation* to obtain a solution having a known concentration of about 0.00004 mg per mL.

Test solution—Transfer about 40 mg of Orbifloxacin, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with *Buffer* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—Inject the *Buffer* as directed for *Procedure* to verify that there are no interfering peaks.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the percentage of related compounds in the portion of Orbifloxacin taken by the formula:

$$20,000(C_S)(r_i / r_S)(1 / F)$$

in which C_S is the concentration, in mg per mL, of orbifloxacin in the *Standard solution*; r_i is the peak area response for each impurity obtained from the *Test solution*; r_S is the peak area response for the orbifloxacin peak obtained from the *Standard solution*; and F is the relative response factor for each impurity, as presented in *Table 1*.

Assay—

Buffer—In a 2-L flask, dissolve about 11.8 g of sodium citrate in 1600 mL of water, and mix. Add 180 mL of acetic acid, and mix. Adjust with 6 N sodium hydroxide to a pH of 3.5, dilute with water to about 2 L, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer*, methanol, and dioxane (86:11:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard stock preparation—Dissolve in *Buffer* an accurately weighed quantity of USP Orbifloxacin RS to obtain a solution having a known concentration of about 0.2 mg per mL.

Standard preparation—Accurately transfer a quantity of *Standard stock preparation*, and dilute with *Buffer* to obtain a solution having a known concentration of about 0.02 mg per mL.

System suitability preparation—Dissolve about 40 mg of methyl 4-aminobenzoate in 2 mL of methanol, and dilute with *Buffer* to 200 mL. Pipet 10.0 mL of this solution and

Table 1

Component/Impurity	Approximate Relative Retention Time	Relative Response Factor (F)	Limit %
<i>cis, cis</i> -1-Cyclopropyl-5,7-bis(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid	0.5	0.36	NMT 0.2
<i>cis</i> -1-Cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-5,6,8-trifluoro-4(1 <i>H</i>)-quinolinone	0.65	0.27	NMT 0.2
7-[(2-Aminopropyl)amino]-1-cyclopropyl-5,6-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid	0.75	0.49	NMT 0.2
Orbifloxacin	1.0	1.00	—
1-Cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid	1.4	0.84	NMT 0.2
<i>cis</i> -1-Cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-5-hydroxy-4-oxo-3-quinolinecarboxylic acid	2.7	0.73	NMT 0.2
<i>cis</i> -1-Cyclopropyl-5-(3,5-dimethyl-1-piperazinyl)-6,7,8-trifluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid	3.6	0.11	NMT 0.2
1-Cyclopropyl-5,6,7,8-tetrafluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid	6.8	0.16	NMT 0.2
Unknown	—	1.0	—
Total known and unknown	—	—	NMT 0.4

10.0 mL of *Standard stock preparation* into a 100-mL volumetric flask. Dilute with *Buffer* to volume, and mix.

Assay preparation—Transfer about 40 mg of Orbifloxacin accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with *Buffer* to volume, and mix. Dilute with *Buffer* an aliquot of the resulting solution to obtain a solution having a known concentration of about 0.02 mg per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 290-nm detector and 4.6-mm × 3.0-cm column that contains 3-μm packing L1. The flow rate is about 1.0 mL per minute. Prior to injecting the *System suitability preparation*, flush the column with approximately 50 mL of a mixture of acetonitrile and water (9:1). Chromatograph the *System suitability preparation*, and record the peak response as directed for *Procedure*: the relative retention times are about 1.3 for methyl 4-aminobenzoate and 1.0 for orbifloxacin; the resolution, R , between methyl 4-aminobenzoate and orbifloxacin is not less than 2; the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatographs, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_{19}H_{20}F_3N_3O_3$ in the portion of Orbifloxacin taken by the formula:

$$2000C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Orbifloxacin RS in the *Standard preparation*; and r_u and r_s are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Orbifloxacin Tablets

» Orbifloxacin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of orbifloxacin ($C_{19}H_{20}F_3N_3O_3$).

Packaging and storage—Preserve in tight containers, and store between 2° and 30°.

Labeling—Label to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Orbifloxacin RS

Identification—

A: Thin-Layer Chromatographic Identification Test (201).

Absorbent: silica gel.

Diluent: a mixture of chloroform, methanol, and glacial acetic acid (8:1:1).

Test solution—Crush 1 Tablet and transfer into a centrifuge tube. Add *Diluent* quantitatively, and mix to obtain a final concentration of about 0.56 mg per mL of orbifloxacin. Centrifuge the solution.

Standard solution—Prepare a solution of USP Orbifloxacin RS in *Diluent* having a concentration of about 0.56 mg per mL.

Application volume: 5 μL

Developing solvent system: a mixture of chloroform, methanol, water, and ammonium hydroxide (18:7:1:0.02).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the

chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 1000 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{19}H_{20}F_3N_3O_3$ dissolved by employing the following procedure.

Standard solution—Prepare a solution of USP Orbifloxacin RS in *Medium* with a final concentration of about $L/100$, where L is the Tablet label claim in mg. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Medium* to volume, and mix.

Test solution—Pass a portion of the solution under test through a suitable 0.8-μm filter, discarding the first 3 mL.

Procedure—Determine the amount of $C_{19}H_{20}F_3N_3O_3$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 291 nm on portions of the *Test solution* in comparison with the *Standard solution* using *Medium* as blank. Calculate the amount of orbifloxacin dissolved by the formula:

$$100,000(A_u / A_s)(C_s / L)$$

in which A_u and A_s are the absorbances obtained with the *Test solution* and the *Standard solution*, respectively; C_s is the concentration, in mg per mL, of orbifloxacin in the *Standard solution*; and L is the Tablet label claim in mg.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{19}H_{20}F_3N_3O_3$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Water Determination, Method 1c (921): between 3.5% and 7.0%.

Test preparation—Accurately weigh 5 Tablets, and transfer into a 50-mL centrifuge tube. Add 25 mL of anhydrous methanol, and cap.

Blank: 25 mL of anhydrous methanol in a 50-mL centrifuge tube.

Procedure—Rotate the *Test preparation* and the *Blank* for 16 hours. Centrifuge. Titrate an equal volume of the *Test preparation* and the *Blank* so that the amount of water titrated will be approximately 1000 μg to 1500 μg.

Chromatographic purity—

Buffer, Mobile phase, Standard preparation, System suitability preparation, and Chromatographic system—Prepare as directed in the *Assay*.

Standard solution—Dilute quantitatively with *Buffer* the *Standard preparation* to obtain a solution having a known concentration of about 0.00004 mg per mL.

Test solution—Transfer 10 Tablets into a volumetric flask. Add *Buffer* to fill the flask about 70%, shake for 2 hours, and sonicate for 5 minutes. Dilute quantitatively, and stepwise if necessary, with *Buffer* to obtain a solution having a concentration of about 0.22 mg per mL. Pass a portion of the solution through a 0.8-μm filter.

Chromatographic system (see *Chromatography* (621))—Inject the *Buffer* as directed for *Procedure* to verify that there are no interfering peaks.

Procedure—Separately inject equal volumes (about 10 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the percentage of related compounds in the portion of Tablets taken by the formula:

$$100(C_s / C_T)(r_i / r_s)(1 / F)$$

Table 1

Component	Relative Retention Time	Relative Response Factor (F)	Limit (%)
<i>cis</i> -1-Cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-5,6,8-trifluoro-4(1 <i>H</i>)-quinolinone	0.57	0.29	NMT 0.5
Orbifloxacin	1.0	1.00	—
<i>cis</i> -1-Cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-5-hydroxy-4-oxo-3-quinolinecarboxylic acid	2.9	0.71	NMT 0.5
All other related compounds and impurities	—	0.11	NMT 0.5
Total known and unknown	—	—	NMT 1

in which C_s is the concentration, in mg per mL, of USP Orbifloxacin RS in the *Standard solution*; C_t is the concentration, in mg per mL, of the *Test solution*; r_i is the peak area response for each impurity obtained from the *Test solution*; r_s is the peak area response for the orbifloxacin peak obtained from the *Standard solution*; and F is the relative response factor for each impurity, as presented in Table 1.

Assay—

Buffer—In a 2-L flask, dissolve about 11.8 g of sodium citrate in 1600 mL of water, and mix. Add 180 mL of glacial acetic acid, and mix. Adjust with 6 N sodium hydroxide to a pH of 3.5, dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer*, methanol, and dioxane (91:6:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard stock preparation—Dissolve an accurately weighed quantity of USP Orbifloxacin RS in *Buffer*, and dilute quantitatively, and stepwise if necessary, with *Buffer* to obtain a solution having a known concentration of about 0.2 mg per mL.

Standard preparation—Accurately transfer a quantity of *Standard stock preparation* and dilute quantitatively, and stepwise if necessary, with *Buffer* to obtain a solution having a known concentration of about 0.02 mg per mL.

System suitability preparation—Dissolve about 40 mg of methyl 4-aminobenzoate in 2 mL of methanol, and dilute with *Buffer* to 200 mL. Pipet 10.0 mL of this solution and 10.0 mL of the *Standard stock preparation* into a 100-mL volumetric flask. Dilute with *Buffer* to volume, and mix.

Assay preparation—Transfer 10 Tablets into a volumetric flask. Add *Buffer* to fill the flask about 70%, shake for 2 hours, and sonicate for 5 minutes. Dilute quantitatively, and stepwise if necessary, with *Buffer* to obtain a solution having a known concentration of about 0.02 mg per mL. Pass a portion of the solution through a 0.8- μ m filter.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 290-nm detector and 4.6-mm \times 3-cm column that contains 3- μ m packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *System suitability preparation*, and record the peak response as directed for *Procedure*: the relative retention times are about 1.3 for methyl 4-aminobenzoate and 1.0 for orbifloxacin; the resolution, R , between methyl 4-aminobenzoate and orbifloxacin is not less than 2; the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

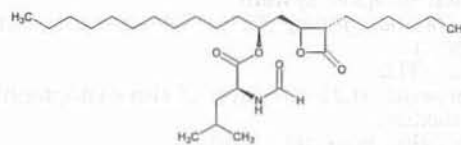
Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of orbifloxacin ($C_{19}H_{20}F_3N_3O_3$) in the portion of Tablets taken by the formula:

$$C(D_u)(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Orbifloxacin RS in the *Standard preparation*; D_u is the dilution factor of the *Assay preparation*, in mL; and r_u and r_s are

the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Orlistat



$C_{29}H_{53}NO_5$ 495.73
L-Leucine, *N*-formyl-, 1-[(3-hexyl-4-oxo-2-oxetanyl)-methyl]dodecyl ester, [2*S*-(2 α (*R**), 3 β)]-;
N-Formyl-L-leucine, ester with (3*S*,4*S*)-3-hexyl-4-[(2*S*)-2-hydroxytridecyl]-2-oxetanone [96829-58-2].

DEFINITION

Orlistat contains NLT 98.0% and NMT 101.5% of orlistat ($C_{29}H_{53}NO_5$), calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

- A. INFRARED ABSORPTION (197K)**
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

[NOTE—Avoid the use of plastic flasks for the preparation or containment of any solution in this analysis.]

Mobile phase: Acetonitrile, phosphoric acid, and water (860:0.05:140)

Standard solution: 0.5 mg/mL of USP Orlistat RS in *Mobile phase*. Inject immediately after preparation or store at 5°.

Sample solution: 0.5 mg/mL of Orlistat in *Mobile phase*. Inject immediately after preparation or store at 5°.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 195

Column: 3.9-mm \times 15-cm; 4- μ m packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of orlistat ($C_{29}H_{53}NO_5$) in the portion of Orlistat taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)
 C_U = concentration of Orlistat in the *Sample solution* (mg/mL)
 Acceptance criteria: 98.0%–101.5% on the anhydrous, solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm • (Official 1-

Jan-2018)

- **LIMIT OF ORLISTAT RELATED COMPOUND A**

Standard solution: 0.1 mg/mL of USP Orlistat Related Compound A RS in acetone

Sample solution: 50 mg/mL of Orlistat in acetone

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: Toluene and ethyl acetate (4:1)

Detection solution: Transfer 2.5 g of phosphomolybdic acid and 1 g of ceric sulfate into a 100-mL volumetric flask, and dissolve in and dilute with methanol to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Remove the plate, and air-dry it thoroughly. Spray the dried plate with *Detection solution*, and place the plate in an oven at 120° for 30 min.

Acceptance criteria: Any secondary spot from the *Sample solution* corresponding to orlistat related compound A is not more intense than the corresponding spot from the *Standard solution* (0.2%).

- **LIMIT OF ORLISTAT RELATED COMPOUND B**

Standard solution: 0.025 mg/mL of USP Orlistat Related Compound B RS in methylene chloride

Sample solution: 50 mg/mL of Orlistat in methylene chloride

Spiked sample solution: 50 mg/mL of Orlistat in *Standard solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm \times 30-m fused silica, coated with a 0.25- μ m G27 stationary phase

Temperatures

Injector: 270°

Detector: 280°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	4	170	—
170	30	300	30

Carrier gas: Helium

Flow rate: 1 mL/min

Split ratio: 20:1

Injection volume: 2 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 10.0%

Analysis

Samples: *Standard solution*, *Sample solution*, and *Spiked sample solution*

Calculate the percentage of orlistat related compound B in the portion of Orlistat taken:

$$\text{Result} = \{r_U/[r_{SP} - r_U \times (C_T/C_U)]\} \times (C_S/C_U) \times 100$$

r_U = peak response of orlistat related compound B from the *Sample solution*

r_{SP} = peak response of orlistat related compound B from the *Spiked sample solution*

C_T = concentration of Orlistat in the *Spiked sample solution* (mg/mL)

C_U = concentration of Orlistat in the *Sample solution* (mg/mL)

C_S = concentration of USP Orlistat Related Compound B RS in the *Standard solution* (mg/mL)

Acceptance criteria: NMT 0.05% of orlistat related compound B is found.

- **ORGANIC IMPURITIES**

[NOTE—Avoid the use of plastic flasks for the preparation or containment of any solution in this analysis.]

Mobile phase, Standard solution, and Sample solution: Prepare as directed in the *Assay*.

System suitability solution: 10 μ g/mL of USP Orlistat RS and 0.1 μ g/mL of USP Orlistat Related Compound C RS in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 195 nm

Column: 3.9-mm \times 15-cm; 4- μ m packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

System suitability

Sample: *System suitability solution*

Suitability requirements

Signal-to-noise ratio: NLT 3 for orlistat related compound C

Relative standard deviation: NMT 10.0% for the orlistat peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity other than orlistat related compound A, orlistat related compound B, orlistat related compound D, orlistat open ring amide, and orlistat related compound E in the portion of Orlistat taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of orlistat from the *Standard solution*

C_S = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)

C_U = concentration of Orlistat in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Formylleucine ^a	0.10	4.0	0.2
Orlistat related compound C	0.13	33	0.05
Orlistat open ring epimer ^b	0.44	1.0	0.2
Orlistat related compound D ^c	0.90	—	see Table 3
Orlistat open ring amide ^{c,d}	0.90	—	see Table 3
Orlistat	1.00	—	—
D-Leucine orlistat ^e	1.18	1.0	0.2
Individual unidentified impurity	—	1.0	0.1
Total impurities ^f	—	—	1.0

^a N-Formyl-L-leucine.^b (2S,3R,5S)-5-[(S)-2-Formylamino-4-methyl-pentanoyloxy]-2-hexyl-3-hydroxy-hexadecanoic acid.^c Coelutes in this LC system, determined using Limits of Orlistat Related Compound D and Orlistat Open Ring Amide.^d N-Formyl-L-leucine (S)-1-[(2S,3S)-2-hydroxy-3-[1-phenyl-R-ethyl-carbomoyl]nonyl]-dodecyl ester.^e N-Formyl-D-leucine (S)-1-[(2S,3S)-3-hexyl-4-oxo-2-oxetanyl]methyl]dodecyl ester or enantiomer.^f Sum of results obtained in the tests for Limit of Orlistat Related Compound A, Limit of Orlistat Related Compound B, Organic Impurities, Limits of Orlistat Related Compound D and Orlistat Open Ring Amide, and Limit of Orlistat Related Compound E.

• LIMITS OF ORLISTAT RELATED COMPOUND D AND ORLISTAT OPEN RING AMIDE

Mobile phase: Methanol and water (830:170)

System suitability solution: 4 mg/mL of USP Orlistat RS and 2.4 µg/mL of USP Orlistat Related Compound D RS in acetonitrile, respectively

Standard solution: 5.0 mg/mL of USP Orlistat RS in acetonitrile

Sample solution: 5.0 mg/mL of Orlistat in acetonitrile

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: 205 nm

Column: 4.0-mm × 25-cm; 5-µm packing L7

Flow rate: 0.6 mL/min

Injection volume: 20 µL

System suitability

Sample: System suitability solution

Suitability requirements

Signal-to-noise ratio: NLT 3 for the orlistat related compound D peak

Relative standard deviation: NMT 10.0% for the orlistat peak

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of orlistat related compound D and orlistat open ring amide in the portion of Orlistat taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of orlistat related compound D or orlistat open ring amide from the Sample solution

r_s = peak response of orlistat from the Standard solution

C_s = concentration of USP Orlistat RS in the Standard solution (µg/mL)

C_u = concentration of Orlistat in the Sample solution (µg/mL)

F = relative response factor (see Table 3)

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Orlistat related compound D	0.94	1.0	0.2
Orlistat	1.00	—	—
Orlistat open ring amide ^a	1.25	4.3	0.1

^a N-Formyl-L-leucine (S)-1-[(2S,3S)-2-hydroxy-3-[1-phenyl-R-ethyl-carbomoyl]nonyl]-dodecyl ester.

• LIMIT OF ORLISTAT RELATED COMPOUND E

Buffer: 0.4 N borate solution, adjusted to a pH of 10.2

Derivatizing agent: o-Phthalaldehyde (OPA) solution.

[NOTE—If unable to obtain commercially, the Derivatizing agent can be prepared as 1% each of 3-mercaptopropionic acid and o-phthalaldehyde in 0.4 M borate buffer solution.]

Solution A: Transfer 4.1 g of sodium acetate trihydrate and 40 mg of ethylenediaminetetraacetic acid (EDTA) into a 1-L volumetric flask. Dissolve in 950 mL of water, and adjust with 0.1 N sodium hydroxide to a pH of 7.2. Dilute with water to volume, add 2.5 mL of tetrahydrofuran, and mix. Filter, and degas.

Solution B: Transfer 2.7 g of sodium acetate trihydrate and 40 mg of EDTA into a 1-L volumetric flask. Dissolve in 200 mL of water, and adjust with 0.1 N sodium hydroxide to a pH of 7.2. Add 800 mL of acetonitrile, filter, and degas.

Mobile phase: See Table 4.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	96.7	3.3
20	60	40
24	0	100
38	0	100
38	96.7	3.3
45	96.7	3.3

Standard solution: Transfer a weighed quantity of about 0.2 mg of USP Orlistat Related Compound E RS into a 20-mL headspace vial. Add 10 mL of 4 N sodium hydroxide, and close the vial. Heat the vial to 100° for 1 h, then allow to cool to room temperature. Transfer 2 mL of the resulting solution into a 50-mL volumetric flask, and dilute with water to volume. To 0.5 mL of this solution add 2.0 mL of Buffer and 0.5 mL of Derivatizing agent.

Sample solution: Proceed as directed for the Standard solution, except use 25 mg of Orlistat to replace the 0.2 mg of USP Orlistat Related Compound E RS.

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: Fluorescence 340 nm (excitation); 450 nm (emission)

Columns

Guard: 2.1-mm × 2-cm; 5-µm packing L1

Analytical: 2.1-mm × 20-cm; 5-µm packing L1

Flow rate: 0.5 mL/min

Injection volume: 20 µL

System suitability

Sample: Standard solution

Suitability requirements

Relative standard deviation: NMT 6.0% for the orlistat related compound E peak

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of orlistat related compound E in the portion of Orlistat taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of orlistat related compound E from the *Sample solution*

r_S = peak response of orlistat related compound E from the *Standard solution*

C_S = concentration of USP Orlistat Related Compound E RS in the *Standard solution* (mg/mL)

C_U = concentration of Orlistat in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2% of orlistat related compound E is found.

SPECIFIC TESTS• **OPTICAL ROTATION, Specific Rotation (781S)**

Sample solution: 30 mg/mL in dehydrated alcohol
Acceptance criteria: Between -48.0° and -51.0° , at 20°

• **WATER DETERMINATION, Method 1c (921):** NMT 0.2%**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers between 2° and 8° .• **USP REFERENCE STANDARDS (11)**

USP Orlistat RS

USP Orlistat Related Compound A RS

(3*S*,4*S*)-3-Hexyl-4-[(2*R*)-2-hydroxytridecyl]-2-oxetanone.
 $C_{22}H_{42}O_3$ 354.57

USP Orlistat Related Compound B RS

Diisopropyl hydrazine-1,2-dicarboxylate.

$C_8H_{16}N_2O_4$ 204.22

USP Orlistat Related Compound C RS

Triphenylphosphine oxide.

$C_{18}H_{15}OP$ 278.28

USP Orlistat Related Compound D RS

N-Formyl-L-leucine (3*S*,4*R*,6*S*)-3-hexyl-2-oxo-6-undecyltetrahydro-2*H*-pyran-4-yl ester.

$C_{29}H_{53}NO_5$ 495.73

USP Orlistat Related Compound E RS

N-Formyl (S)-isoleucine (S)-1-[[[(2*S*,3*S*)-3-hexyl-4-oxo-2-oxetanyl]methyl]dodecyl ester.

$C_{29}H_{53}NO_5$ 495.73

min, and dilute with *Mobile phase* to volume. Pass a portion of this solution through a filter of $0.45\text{-}\mu\text{m}$ or finer pore size, discarding the first few mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: 195 nm

Column: 3.9-mm \times 15-cm; packing L1

Flow rate: 1.0 mL/min

Injection size: 20 μL

System suitability

Sample: *Standard solution*

System suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of orlistat ($C_{29}H_{53}NO_5$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of orlistat in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: 3% Sodium lauryl sulfate and 0.5% sodium chloride in water. To each 10 L of media add 1–2 drops of *n*-octanol, and adjust with phosphoric acid to a pH of 6.0; 900 mL.

Apparatus 2: 75 rpm, with coil wire sinker

Time: 45 min

Mobile phase: Acetonitrile and water (860:140)

Standard solution: Transfer about 13 mg of USP Orlistat RS to a 100-mL volumetric flask. Dissolve in 2 mL of acetonitrile, and dilute with *Medium* to volume.

Sample solution: Pass a portion of the solution under test through a suitable filter of $0.2\text{-}\mu\text{m}$ pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode, Detector, and Column: Proceed as directed in the *Assay*.

Flow rate: 2.0 mL/min

Injection size: 50 μL

System suitability

Sample: *Standard solution*

System suitability requirements

Relative standard deviation: NMT 2.0%

Calculate the percentage of the labeled amount of orlistat ($C_{29}H_{53}NO_5$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of orlistat is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**IMPURITIES****Organic Impurities**• **PROCEDURE**

Mobile phase, Standard solution, and Sample solution: Prepare as directed in the *Assay*.

System suitability solution: 0.025 mg/mL of USP Orlistat Related Compound D RS in *Mobile phase*. Transfer

Orlistat Capsules**DEFINITION**

Orlistat Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of orlistat ($C_{29}H_{53}NO_5$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Mobile phase: Acetonitrile, phosphoric acid, and water (860:0.05:140)

Standard solution: 0.6 mg/mL of USP Orlistat RS in *Mobile phase*

Sample solution: Transfer the contents of NLT 10 Capsules into a suitable container, weigh, and mix. Transfer an accurately weighed portion of the powder, equivalent to 120 mg of orlistat, into a 200-mL volumetric flask. Add 140 mL of *Mobile phase*, and sonicate for 1 min. Shake the resulting solution mechanically for 15

1 mL of this solution to a 50-mL volumetric flask, and dilute with *Standard solution* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

System suitability

Sample: *System suitability solution*

System suitability requirements

Resolution: NLT 1.4 between USP Orlistat RS and USP Orlistat Related Compound D RS

Relative standard deviation: NMT 2.0% for the orlistat peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each individual impurity in the *Sample solution*

r_s = peak response of orlistat in the *Standard solution*

C_s = concentration of USP Orlistat RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of orlistat in the *Sample solution* (mg/mL)

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Orlistat open-ring epimer ^a	0.45	1.0	1.5
Orlistat open ring ^b	0.5	1.0	0.3
Orlistat related compound D	0.9	1.0	1.0
Orlistat	1.0	—	—
Hexyl undecyl pyranone ^c	2.0	1.0	0.2
Henicosenyl leucinate ^d	4.7	2.3	0.3
Any other identified impurity	—	—	0.3
Individual unidentified impurity	—	1.0	0.2
Total impurities	—	—	3.0

^a (2S,3R,5S)-5-[(N-Formyl-L-leucyl)oxy]-2-hexyl-3-hydroxyhexadecanoic acid.

^b (2S,3S,5S)-5-[(N-Formyl-L-leucyl)oxy]-2-hexyl-3-hydroxyhexadecanoic acid.

^c (S)-3-Hexyl-5,6-dihydro-6-undecyl-2H-pyran-2-one.

^d (S)-[(S,E)-Henicos-7-en-10-yl] N-formyl-L-leucinate.

ADDITIONAL REQUIREMENTS

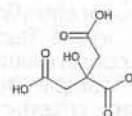
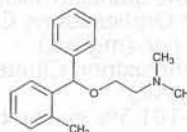
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at 25°, excursions permitted between 15° and 30°.

• USP REFERENCE STANDARDS (11)

USP Orlistat RS

USP Orlistat Related Compound D RS

Orphenadrine Citrate



$C_{18}H_{23}NO \cdot C_6H_8O_7$ 461.50

Ethanamine, N,N-dimethyl-2-[(2-methylphenyl)phenylmethoxy]-, (±)-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1); (±)-N,N-Dimethyl-2-[(o-methyl-α-phenylbenzyl)oxy]ethylamine citrate (1:1) [4682-36-4].

DEFINITION

Orphenadrine Citrate contains NLT 98.0% and NMT 101.5% of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **C. IDENTIFICATION TESTS—GENERAL, Citrate** (191): Meets the requirements

ASSAY

• PROCEDURE

Buffer: 5.8 g/L of monobasic ammonium phosphate in water. Adjust with ammonium hydroxide or phosphoric acid to a pH of 7.9.

Mobile phase: Methanol, acetonitrile, and *Buffer* (45:15:40)

System suitability solution: 0.01 mg/mL each of USP Orphenadrine Related Compound B RS, USP Orphenadrine Related Compound C RS, and USP Methylbenzhydrol RS and 1.0 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Standard solution: 1.0 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Sample solution: 1.0 mg/mL of Orphenadrine Citrate in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

Run time: NLT 2.5 times the retention time of orphenadrine

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between orphenadrine related compound B and C; NLT 3.0 between orphenadrine related compound C and methylbenzhydrol, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the portion of Orphenadrine Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)
 C_U = concentration of Orphenadrine Citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

- **ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—If methyl orphenadrine is a known manufacturing impurity, *Procedure 1* and the test for *Isomer Content* are recommended. If diphenhydramine and didesmethyl orphenadrine are known manufacturing process impurities, *Procedure 2* is recommended.]

Buffer, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.001 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Sensitivity solution: 0.5 µg/mL of USP Orphenadrine Citrate RS, from the *Standard solution*, in *Mobile phase*

Sample solution: 1 mg/mL of Orphenadrine Citrate in *Mobile phase*

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between orphenadrine related compound B and C; NLT 3.0 between orphenadrine related compound C and 2-methylbenzhydrol, *System suitability solution*

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Orphenadrine Citrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- r_U = peak area of each impurity from the *Sample solution*
 r_S = peak area of Orphenadrine Citrate from the *Standard solution*
 C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)
 C_U = concentration of the *Sample solution* (mg/mL)
 F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citric acid ^a	0.14	—	—
Orphenadrine related compound B	0.25	1.3	0.1
Orphenadrine related compound C	0.39	1.0	0.3
Methylbenzhydrol	0.51	2.4	0.1
Diphenhydramine	0.69	1.0	0.3
Orphenadrine	1.0	—	—
Methyl orphenadrine ^b	1.54	1.9	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities ^c	—	—	0.5

^a Counter ion peak; not to be reported; not to be included in total impurities.

^b 2-(Di-*o*-tolylmethoxy)-*N,N*-dimethylethan-1-amine.

^c Excluding orphenadrine related compound E and orphenadrine related compound F from the *Isomer Content* test.

- **ORGANIC IMPURITIES, PROCEDURE 2**

[NOTE—If didesmethyl orphenadrine is a known manufacturing process impurity, *Procedure 2* is recommended. The labeling indicates that the article complies with *Organic Impurities, Procedure 2*.]

System suitability solution: 0.3 mg/mL each of USP Orphenadrine Citrate RS, USP Diphenhydramine Citrate RS, USP Methylbenzhydrol RS, USP Orphenadrine Related Compound E RS, and USP Orphenadrine Related Compound F RS in toluene prepared as follows. Dissolve 6 mg each of the USP Reference Standards in 10 mL of water. Add 0.2 mL of ammonium hydroxide and shake with 3 mL of toluene. Separate the organic layer. Repeat the extraction of the aqueous layer two more times with 3 mL of toluene. To the combined organic layer add anhydrous sodium sulfate, shake, and filter. Evaporate the filtrate at NMT 50° using a rotary evaporator. Dissolve the residue in 20 mL of toluene.

Sample solution: 25 mg/mL of Orphenadrine Citrate in toluene prepared as follows. Dissolve 500 mg of Orphenadrine Citrate in 50 mL of water. Add 2 mL of ammonium hydroxide and shake with 10 mL of toluene. Separate the organic layer. Repeat the extraction of the aqueous layer two more times with 10 mL of toluene. To the combined organic layers add anhydrous sodium sulfate, shake, and filter. Evaporate the filtrate at NMT 50° using a rotary evaporator. Dissolve the residue in 20 mL of toluene.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 60-m; 1-µm thick coating of phenyl methylpolysiloxane, packing G27

Carrier gas: Helium at 1 mL/min

Temperatures

Injection port: 290°

Detector: 290°

Column: 240°

Injection volume: 2 µL

Injection type: Split ratio, 1:25

Run time: 1.3 times the retention time of orphenadrine

System suitability

Sample: *System suitability solution*

[NOTE—Relative retention times for the peaks are given in *Table 2*.]

Suitability requirements

Resolution: NLT 1.5 between orphenadrine related compound E and orphenadrine; NLT 2.0 between orphenadrine and orphenadrine related compound F

Analysis**Sample:** *Sample solution*

Calculate the sum of the percentage of any individual impurity in the portion of Orphenadrine Citrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 r_U = peak response of the impurity from the *Sample solution* r_T = sum of all peak responses from the *Sample solution***Acceptance criteria:** See Table 2. Disregard any peak less than 0.05%.**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (% w/w)
Methylbenzophenone ^a	0.5	0.3
Methylbenzhydrol	0.6	0.3
Diphenhydramine	0.8	0.3
Didesmethyl orphenadrine ^b	0.9	0.3
Orphenadrine related compound E	0.98	0.3
Orphenadrine	1.0	—
Orphenadrine related compound F	1.1	0.3
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

^a Phenyl(o-tolyl)methanone.^b 2-[Phenyl(o-tolyl)methoxy]ethanamine.**• ISOMER CONTENT**If methyl orphenadrine is a known manufacturing impurity, the test for *Isomer Content* is to be performed.

System suitability solution: 0.3 mg/mL each of USP Orphenadrine Citrate RS, USP Orphenadrine Related Compound E RS, and USP Orphenadrine Related Compound F RS in toluene prepared as follows. Dissolve 6 mg each of the USP Reference Standards in 10 mL of water. Add 0.2 mL of ammonium hydroxide and shake with 3 mL of toluene. Separate the organic layer. Repeat the extraction of the aqueous layer two more times with 3 mL of toluene. To the combined organic layer add anhydrous sodium sulfate, shake, and filter. Evaporate the filtrate at NMT 50° using a rotary evaporator. Dissolve the residue in 20 mL of toluene.

Sample solution and Chromatographic system: Proceed as directed in *Organic Impurities, Procedure 2*.**System suitability****Sample:** *System suitability solution*

[NOTE—The relative retention times of orphenadrine related compound E, orphenadrine, and orphenadrine related compound F are about 0.98, 1.0, and 1.1, respectively.]

Suitability requirements**Resolution:** NLT 1.5 between orphenadrine related compound E and orphenadrine; NLT 2.0 between orphenadrine and orphenadrine related compound F**Analysis****Sample:** *Sample solution*

Calculate the percentage of orphenadrine related compound E and orphenadrine related compound F in the portion of Orphenadrine Citrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 r_U = peak response of orphenadrine related compound E or orphenadrine related compound F from the *Sample solution* r_T = sum of all peak responses from the *Sample solution***Acceptance criteria:** NMT 0.3% each of orphenadrine related compound E and orphenadrine related compound F**SPECIFIC TESTS****• LOSS ON DRYING (731)****Analysis:** Dry at 105° for 3 h.**Acceptance criteria:** NMT 0.5%**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.**• LABELING:** The label states with which *Organic Impurities* procedure the article complies if *Organic Impurities, Procedure 1*, is not used.**• USP REFERENCE STANDARDS (11)**

USP Diphenhydramine Citrate RS

USP Methylbenzhydrol RS

2-Methylbenzhydrol;

Also known as Phenyl(o-tolyl)methanol.

C₁₄H₁₄O 198.26

USP Orphenadrine Citrate RS

USP Orphenadrine Related Compound B RS

N-Ethyl-N,N-dimethyl-2-[phenyl(o-tolyl)methoxy]ethanaminium chloride.

C₂₀H₂₈ClNO 333.90

USP Orphenadrine Related Compound C RS

N-Methyl-2-[phenyl(o-tolyl)methoxy]ethanamine hydrochloride.

C₁₇H₂₁NO · HCl 291.82

USP Orphenadrine Related Compound E RS

N,N-Dimethyl-2-[phenyl(m-tolyl)methoxy]ethanamine.

C₁₈H₂₃NO 269.38

USP Orphenadrine Related Compound F RS

N,N-Dimethyl-2-[phenyl(p-tolyl)methoxy]ethanamine.

C₁₈H₂₃NO 269.38**Orphenadrine Citrate Injection****DEFINITION**Orphenadrine Citrate Injection is a sterile solution of Orphenadrine Citrate in Water for Injection, prepared with the aid of Sodium Hydroxide. It contains NLT 93.0% and NMT 107.0% of the labeled amount of orphenadrine citrate (C₁₈H₂₃NO · C₆H₈O₇).**IDENTIFICATION****• A.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.**• B. IDENTIFICATION TESTS—GENERAL (191), Citrate:** Meets the requirements**ASSAY****• PROCEDURE****Buffer:** 5.8 g/L of monobasic ammonium phosphate in water. Adjust with ammonium hydroxide or phosphoric acid to a pH of 7.9 ± 0.05.**Mobile phase:** Methanol, acetonitrile, and *Buffer* (45:15:40)**System suitability solution:** 0.01 mg/mL each of USP Orphenadrine Related Compound B RS, USP Orphenadrine Related Compound C RS, USP Methylbenzhydrol RS, and 0.9 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase***Standard solution:** 0.9 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase***Sample solution:** Nominally 0.9 mg/mL of orphenadrine citrate from a known volume of the Injection con-

taining NLT 90 mg of orphenadrine citrate in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

Run time: NLT 2.5 times the retention time of orphenadrine

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between orphenadrine related compound B and orphenadrine related compound C; NLT 3.0 between orphenadrine related compound C and methylbenzhydrol

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the portion of the injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

IMPURITIES

• ORGANIC IMPURITIES

Buffer, *Mobile phase*, *System suitability solution*, *Sample solution*, and *Chromatographic system*: Proceed as directed in the *Assay*.

Standard solution: 0.002 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*

Sensitivity solution: 0.001 mg/mL of USP Orphenadrine Citrate RS from the *Standard solution* in *Mobile phase*

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 3.0 between orphenadrine related compound B and orphenadrine related compound C; NLT 3.0 between orphenadrine related compound C and methylbenzhydrol

Tailing factor: NMT 2, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each degradation product from the *Sample solution*

r_s = peak response of orphenadrine from the *Standard solution*

C_s = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (% w/w)
Citric acid ^a	0.14	—	—
Orphenadrine related compound B	0.25	1.3	0.2
Orphenadrine related compound C	0.39	1.0	0.2
Methylbenzhydrol	0.51	2.4	0.2
Orphenadrine	1.0	—	—
Methyl orphenadrine ^b	1.54	1.9	0.2
Any individual unspecified degradation product	—	1.0	0.20
Total degradation products	—	—	4.0

^a Counter ion peak; not to be reported; not to be included in total impurities.

^b 2-(Di-*o*-tolylmethoxy)-*N,N*-dimethylethan-1-amine.

SPECIFIC TESTS

- **PH (791):** 5.0–6.0
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 5.8 USP Endotoxin Units/mg of orphenadrine citrate
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
 - USP Endotoxin RS
 - USP Methylbenzhydrol RS
 - 2-Methylbenzhydrol; Also known as phenyl(*o*-tolyl)methanol. $C_{14}H_{14}O$ 198.26
 - USP Orphenadrine Citrate RS
 - USP Orphenadrine Related Compound B RS
 - N*-Ethyl-*N,N*-dimethyl [2-(methylbenzhydryloxy)ethyl]ammonium chloride; also known as *N*-ethyl-*N,N*-dimethyl-2-[phenyl(*o*-tolyl)methoxy]ethanaminium chloride. $C_{20}H_{28}ClNO$ 333.90
 - USP Orphenadrine Related Compound C RS
 - N*-Methyl [2-(2-methylbenzhydryloxy)ethyl]amine hydrochloride; also known as *N*-methyl-2-[phenyl(*o*-tolyl)methoxy]ethanamine hydrochloride. $C_{17}H_{21}NO \cdot HCl$ 291.82

Orphenadrine Citrate Extended-Release Tablets

DEFINITION

Orphenadrine Citrate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$).

IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**PROCEDURE**

Buffer: 5.8 g/L of monobasic ammonium phosphate.

Adjust with phosphoric acid to a pH of 3.2.

Mobile phase: Acetonitrile and *Buffer* (40:60)

System suitability solution: 0.1 mg/mL of USP Orphenadrine Citrate RS and 0.01 mg/mL each of USP Orphenadrine Related Compound B RS and USP Orphenadrine Related Compound C RS, in *Mobile phase*

Standard solution: 0.1 mg/mL of USP Orphenadrine Citrate RS

Sample stock solution: Nominally 0.5 mg/mL of orphenadrine citrate prepared as follows. Transfer a quantity of powder equivalent to NLT 100 mg of orphenadrine citrate, from finely powdered Tablets (NLT 20), to a suitable volumetric flask. Add 50% of the flask volume of *Mobile phase*. Sonicate for 5 min and shake for 15 min. Dilute with *Mobile phase* to volume. Pass through a suitable filter.

Sample solution: 0.1 mg/mL of orphenadrine citrate in *Mobile phase* from *Sample stock solution*

Chromatographic system
(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 3.9-mm × 30-cm; 10-μm L1 packing

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between orphenadrine and orphenadrine related compound C; NLT 2.0 between orphenadrine citrate and orphenadrine related compound B

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of orphenadrine citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS**DISSOLUTION (711)****Test 1**

Medium: Water; 900 mL, deaerated

Apparatus 2: 50 rpm

Times: 1, 2, 6, and 12 h

Buffer: 5.8 g/L of monobasic ammonium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (40:60). Adjust with phosphoric acid to a pH of 3.2 ± 0.1 .

Standard stock solution: 1 mg/mL of USP Orphenadrine Citrate RS in *Mobile phase*. Sonication may be used to promote dissolution.

Standard solution: 0.1 mg/mL of USP Orphenadrine Citrate RS in *Medium* from a suitable volume of *Standard stock solution* and *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter with 0.45-μm pore size and discard the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 3.9-mm × 30-cm; 10-μm packing L1

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis:

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved in the portion of the sample withdrawn at each time point (i) (mg/mL):

$$C_i = (r_U/r_S) \times C_S$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_3)] + (C_1 \times V_3)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{C_3 \times [V - (2 \times V_3)] + [(C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{C_4 \times [V - (3 \times V_3)] + [(C_3 + C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

C_i = concentration of orphenadrine citrate in the portion of sample withdrawn at time point (i) (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_3 = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 1*.

Table 1

Time point (i)	Time (h)	Amount Dissolved (%)
1	1	10–40
2	2	30–50
3	6	50–80
4	12	NLT 80

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 1, 4, and 12 h

Standard solution: 0.02 mg/mL of USP Orphenadrine Citrate RS in *Medium*

Sample solution: Withdraw 10 mL of the solution under test from each vessel at each specified time point. Replace 10 mL of *Medium* in each vessel. Pass a portion of the solution under test through a suitable filter having a porosity of 0.45 μm. Transfer 1.0 mL of

the filtrate to a 50-mL volumetric flask, and dilute with Medium to volume.

Blank: Medium

Instrumental conditions

Mode: UV 210 nm

Analysis

Samples: Standard solution and Sample solution

Calculate the concentration (C_i) of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result} = (A_U/A_S) \times C_S \times D_S \times (1/L) \times V \times 100$$

A_U = absorbance of the Sample solution

A_S = absorbance of the Standard solution

C_S = concentration of the Standard solution (mg/mL)

D_S = dilution factor of the Sample solution, 50

L = label claim (mg/Tablet)

V = volume of Medium, 900 mL

Calculate the percentage of the labeled amounts (Q) of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = [(C_3 \times V) + [(C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

C_i = concentration of orphenadrine citrate in the portion of sample withdrawn at time point (i) (mg/mL)

V = volume of Medium, 900 mL

L = label claim (mg/Tablet)

V_3 = volume of the Sample solution withdrawn at each time point (mL)

Tolerances: See Table 2.

Table 2

Time point (i)	Time (h)	Amount Dissolved (%)
1	1	10–40
2	4	40–70
3	12	NLT 80

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Buffer, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Sample: Sample solution

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (1/F) \times 100$$

r_U = peak response of each degradation product from the Sample solution

r_S = peak response of orphenadrine from the Sample solution

F = relative response factor for each degradation product (see Table 3)

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citric acid ^a	0.4	—	—
Orphenadrine related compound C	0.9	1.5	0.5
Orphenadrine citrate	1	—	—
Orphenadrine related compound B	1.3	1.3	0.5
2-Methylbenzhydrol ^b	2.1	2.1	0.5
2-Methylbenzophenone ^c	4	1.0	0.5
Any individual unspecified degradation product	—	1.0	0.10
Total degradation products	—	—	1.5

^aThe peak is due to counter ion and is not to be reported or included in total degradation products.

^bAlso known as phenyl(o-tolyl)methanol.

^cAlso known as phenyl(o-tolyl)methanone.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one Dissolution test is given, the labeling states the Dissolution test used only if Test 1 is not used.
- **USP REFERENCE STANDARDS (11)**
 - USP Orphenadrine Citrate RS
 - USP Orphenadrine Related Compound B RS
 - N-Ethyl-N,N-dimethyl [2-(2-methylbenzhydryloxy)ethyl]ammonium chloride; also known as N-ethyl-N,N-dimethyl-2-[phenyl(o-tolyl)methoxy]ethanaminium chloride. $C_{20}H_{28}ClNO$ 333.90
 - USP Orphenadrine Related Compound C RS
 - N-Methyl [2-(2-methylbenzhydryloxy)ethyl]amine hydrochloride; also known as N-methyl-2-[phenyl(o-tolyl)methoxy]ethanamine hydrochloride. $C_{17}H_{22}ClNO$ 291.82

Orphenadrine Citrate, Aspirin, and Caffeine Tablets

DEFINITION

Orphenadrine Citrate, Aspirin, and Caffeine Tablets contain NLT 90.0% and NMT 110.0% each of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$), aspirin ($C_9H_8O_4$), and caffeine ($C_8H_{10}N_4O_2$).

IDENTIFICATION

- **A.** The retention times of the orphenadrine, aspirin, and caffeine peaks in the chromatogram of the Sample solution correspond to those of the orphenadrine, aspirin, and caffeine peaks in the chromatogram of the Standard solution, as obtained in the Orphenadrine Citrate and Aspirin and Caffeine tests in the Assay.

ASSAY• **ORPHENADRINE CITRATE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.6. To each L of this solution add 5.8 g of sodium dodecyl sulfate, and dissolve.

Mobile phase: Acetonitrile and *Buffer* (50:50)

Diluent: Methanol and glacial acetic acid (92:8)

Standard solution: 0.1 mg/mL of USP Orphenadrine Citrate RS prepared as follows. Transfer the weighed amount of standard to a suitable volumetric flask with the aid of methanol. Add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional shaking for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume. Filter a portion through a suitable filter of 0.45- μ m pore size, discarding at least the first 3 mL of the filtrate.

Sample stock solution: Nominally 0.5 mg/mL of orphenadrine citrate from Tablets (NLT 5), prepared as follows. To the volumetric flask containing the Tablets, add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional hand shaking for NLT 15 min. Shake mechanically for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume. [NOTE—This solution also contains 7.7 mg/mL of aspirin and 0.6 mg/mL of caffeine.]

Sample solution: Nominally 0.1 mg/mL of orphenadrine citrate by diluting a suitable portion of the *Sample stock solution* with *Diluent*. Filter a portion through a suitable filter of 0.45- μ m pore size, discarding at least the first 3 mL of the filtrate. [NOTE—This solution also contains 1.5 mg/mL of aspirin and 0.1 mg/mL of caffeine.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 257 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L1

Flow rate: 2 mL/min

Injection volume: 15 μ L

Run time: 1.5 times the retention time of orphenadrine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of orphenadrine citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

• **ASPIRIN AND CAFFEINE**

Buffer: Dissolve 0.8 g/L of hexanesulfonic acid sodium salt in water. Adjust with glacial acetic acid to a pH of 3.0.

Mobile phase: Methanol and *Buffer* (40:60)

Diluent: Methanol and glacial acetic acid (92:8)

Standard solution: 1.5 mg/mL of USP Aspirin RS and 0.1 mg/mL of USP Caffeine RS prepared as follows. Transfer the weighed amount of standards to a suitable volumetric flask with the aid of methanol. Add glacial acetic acid to fill 8% of final volume and methanol to

fill 75% of final volume. Sonicate with occasional hand shaking for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume. Filter a portion through a suitable filter of 0.45- μ m pore size, discarding at least the first 3 mL of the filtrate.

Sample stock solution: Nominally 7.7 mg/mL of aspirin from Tablets (NLT 5), prepared as follows. To the volumetric flask containing the Tablets, add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional hand shaking for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume. [NOTE—This solution also contains 0.6 mg/mL of caffeine and 0.5 mg/mL of orphenadrine citrate.]

Sample solution: Nominally 1.5 mg/mL of aspirin by diluting a suitable portion of the *Sample stock solution* with *Diluent*. Filter a portion through a suitable filter of 0.45- μ m pore size, discarding at least the first 3 mL of the filtrate. [NOTE—This *Sample solution* also contains 0.1 mg/mL of caffeine and 0.1 mg/mL of orphenadrine citrate.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L1

Flow rate: 1.2 mL/min

Injection volume: 15 μ L

Run time: 3 times the retention time of aspirin

System suitability

[NOTE—The relative retention time for caffeine and for aspirin is 0.70 and 1.0, respectively.]

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between caffeine and aspirin peaks

Relative standard deviation: NMT 2.0% each for both the caffeine and aspirin peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of caffeine ($C_8H_{10}N_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of caffeine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% each of aspirin and caffeine

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 45 min for aspirin and caffeine; 60 min for orphenadrine

Separate dissolution baths must be run for the different time points.

Orphenadrine citrate

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.6. To each L of this solution add 5.8 g of sodium dodecyl sulfate, and dissolve.

Mobile phase: Acetonitrile and *Buffer* (50:50)

Standard solution: (L/900) mg/mL of USP Orphenadrine Citrate RS in *Medium*, where L is the label claim in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 257 nm

Column: 3.9-mm × 30-cm; 10-μm packing L1

Flow rate: 2 mL/min

Injection volume: 100 μL

Run time: 1.2 times the retention time of orphenadrine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 3.0%

Calculate the percentage (Q) of the labeled amount of orphenadrine citrate ($C_{18}H_{23}NO \cdot C_6H_8O_7$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response for orphenadrine from the *Sample solution*

r_S = peak response for orphenadrine from the *Standard solution*

C_S = concentration of USP Orphenadrine Citrate RS in the *Standard solution* (mg/mL)

L = label claim for orphenadrine (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of orphenadrine is dissolved in 60 min.

Aspirin and caffeine

Buffer: 0.8 g/L of hexanesulfonic acid sodium salt in water

Mobile phase: Methanol and *Buffer* (40:60). Adjust with glacial acetic acid to a pH of 3.0.

Standard stock solution: 1.0 mg/mL of USP Aspirin RS, 0.1 mg/mL of USP Caffeine RS, and 0.1 mg/mL of USP Salicylic Acid RS prepared as follows. Transfer the weighed amount of standards to a suitable volumetric flask with the aid of methanol. Add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional shaking for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume.

Standard solution: Dilute a suitable volume of the *Standard stock solution* with methanol to obtain a final concentration of about 0.25 mg/mL of USP Aspirin RS and 0.025 mg/mL each of USP Caffeine RS and USP Salicylic acid RS.

Sample solution: At the time specified, withdraw the solution under test and pass through a suitable filter. If necessary, dilute the filtrate with methanol to obtain a final concentration similar to that of the *Standard solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-μm packing L1

Flow rate: 1.2 mL/min

Injection volume: 15 μL

Run time: 2 times the retention time of aspirin

System suitability

[NOTE—The relative retention times for caffeine, aspirin, and salicylic acid are 0.70, 1.0, and 1.3, respectively.]

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0% for both the caffeine and aspirin peaks

Calculate the percentage (Q) of the labeled amount of aspirin ($C_9H_8O_4$) or caffeine ($C_8H_{10}N_4O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

r_U = peak response for aspirin or caffeine from the *Sample solution*

r_S = peak response for aspirin or caffeine from the *Standard solution*

C_S = concentration of USP Aspirin RS or USP Caffeine RS in the *Standard solution* (mg/mL)

L = label claim for aspirin or caffeine (mg/Tablet)

D = dilution factor, if applicable

V = volume of *Medium*, 900 mL

Calculate the percentage of salicylic acid ($C_7H_6O_3$), relative to the labeled amount of aspirin, dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response for salicylic acid from the *Sample solution*

r_S = peak response for salicylic acid from the *Standard solution*

C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

L = label claim for aspirin (mg/Tablet)

D = dilution factor

M_{r1} = molecular weight of aspirin, 180.16

M_{r2} = molecular weight of salicylic acid, 138.12

V = volume of *Medium*, 900 mL

The percentage of salicylic acid dissolved should be added to the percentage of aspirin dissolved.

Tolerances: NLT 70% (Q) of the labeled amounts of aspirin and caffeine is dissolved in 45 min.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES• **LIMIT OF 2-METHYLBENZHYDROL**

Buffer: 6.8 g/L of monobasic potassium phosphate in water

Mobile phase: To 1 L of *Buffer* add 5.8 g of sodium dodecyl sulfate and 1 L of acetonitrile. Adjust with phosphoric acid to a pH of 3.6.

Standard solution: 7.5 μg/mL of USP 2-Methylbenzhydrol RS in methanol

Sample solution: Nominally 1.5 mg/mL of orphenadrine citrate in methanol from a portion of finely powdered Tablets (NLT 20 Tablets). [NOTE—This *Sample solution* also contains 0.6 mg/mL of caffeine and 0.5 mg/mL of orphenadrine citrate.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 213 nm

Column: 4.6-mm × 150-mm; 5-μm packing L1

Flow rate: 1.2 mL/min

Injection volume: 10 μL

Run time: 2.5 times the retention time of 2-methylbenzhydrol

System suitability

Sample: *Standard solution*

Suitability requirements:

Tailing factor: NMT 1.5

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 2-methylbenzhydrol in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP 2-Methylbenzhydrol RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of orphenadrine citrate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

• ORGANIC IMPURITIES

Buffer: 0.8 g/L of sodium 1-hexanesulfonate in water. Adjust with glacial acetic acid to a pH of 3.0.

Mobile phase: Methanol and *Buffer* (40:60)

Diluent: Methanol and glacial acetic acid (92:8)

System suitability solution: 0.6 mg/mL of USP Salicylic Acid RS, 1.5 mg/mL of USP Aspirin RS, and 0.1 mg/mL of USP Caffeine RS prepared as follows.

Transfer the weighed amount of standards to a suitable volumetric flask with the aid of methanol. Add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional hand shaking for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume. Filter a portion through a suitable filter of 0.45- μ m pore size, discarding at least the first 3 mL of the filtrate.

Standard solution: 0.04 mg/mL of USP Salicylic Acid RS prepared as follows. Transfer the weighed amount of standard to a suitable volumetric flask with the aid of methanol. Add glacial acetic acid to fill 8% of final volume, and dilute with methanol to volume. Filter a portion through a suitable filter of 0.45- μ m pore size, discarding at least the first 3 mL of the filtrate.

Sample stock solution: Nominally 7.7 mg/mL of aspirin from Tablets (NLT 5), prepared as follows. To the volumetric flask containing the Tablets, add glacial acetic acid to fill 8% of final volume and methanol to fill 75% of final volume. Sonicate with occasional hand shaking for NLT 15 min. Allow the solution to equilibrate to room temperature. Dilute with methanol to volume. [NOTE—This solution also contains 0.5 mg/mL of orphenadrine citrate and 0.6 mg/mL of caffeine.]

Sample solution: Nominally 1.5 mg/mL of aspirin by diluting a suitable portion of the *Sample stock solution* with *Diluent*. Filter a portion through a suitable filter of 0.45- μ m pore size, discarding at least the first 3 mL of the filtrate. [NOTE—This *Sample solution* also contains 0.1 mg/mL of orphenadrine citrate and 0.1 mg/mL of caffeine.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L1

Flow rate: 1.2 mL/min

Injection volume: 25 μ L

Run time: 3 times the retention time of the aspirin peak

System suitability

[NOTE—Refer to *Table 1* for relative retention times.]

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between the caffeine and aspirin peaks and NLT 1.5 between the aspirin and salicylic acid peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% each for aspirin and caffeine peaks, *System suitability solution*; NMT 3.0% for salicylic acid, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of salicylic acid in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Calculate the percentage of other organic impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = sum of the areas of all other organic impurities from the *Sample solution*

r_T = sum of the areas of all peaks from the *Sample solution*

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Caffeine	0.70	—
Aspirin	1.0	—
Salicylic acid	1.3	3.0
Total impurities ^a	—	2.0

^a Does not include salicylic acid and 2-methylbenzhydrol.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Aspirin RS

USP Caffeine RS

USP 2-Methylbenzhydrol RS

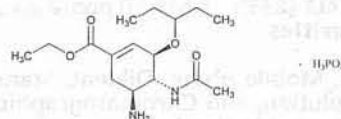
2-Methylbenzhydrol.

$C_{14}H_{14}O$ 198.26

USP Orphenadrine Citrate RS

USP Salicylic Acid RS

Oseltamivir Phosphate



$C_{16}H_{28}N_2O_4 \cdot H_3PO_4$ 410.40
 [3*R*-(3 α ,4 β ,5 α)]-Ethyl 4-(acetamino)-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate phosphate (1:1);
 Ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate, phosphate (1:1)
 [204255-11-8].

DEFINITION

Oseltamivir Phosphate contains NLT 98.0% and NMT 101.5% of $C_{16}H_{28}N_2O_4 \cdot H_3PO_4$, calculated on the anhydrous basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION** (197M)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: Dissolve 6.8 g of potassium dihydrogen phosphate in 980 mL of water. Adjust with 1 M potas-

sium hydroxide solution to a pH of 6.0, and dilute with water to 1 L.

Mobile phase: Methanol, acetonitrile, and *Solution A* (245:135:620)

Diluent: Methanol, acetonitrile, and water (245:135:620)

Standard solution: 1 mg/mL of USP Oseltamivir Phosphate RS in *Diluent*

Sample solution: 1 mg/mL of Oseltamivir Phosphate in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 207 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 50°

Flow rate: 1.2 mL/min

Injection size: 15 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{16}H_{28}N_2O_4 \cdot H_3PO_4$ in the portion of Oseltamivir Phosphate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Oseltamivir Phosphate RS in the *Standard solution* (mg/mL)

C_u = concentration of Oseltamivir Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.5% on the anhydrous basis

IMPURITIES

Inorganic Impurities

Delete the following:

- **HEAVY METALS** (231): NMT 10 ppm • (Official 1-Jan-2018)

Organic Impurities

• PROCEDURE 1

Solution A, Mobile phase, Diluent, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Oseltamivir Phosphate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each individual impurity from the *Sample solution*

r_s = peak response of oseltamivir phosphate from the *Standard solution*

C_s = concentration of USP Oseltamivir Phosphate RS in the *Standard solution* (mg/mL)

C_u = concentration of Oseltamivir Phosphate in the *Sample solution* (mg/mL)

F = relative response factor from *Impurity Table 1*

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.7%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oseltamivir acid ^a	0.17	1.4	0.3
Oseltamivir phenol ^b	0.51	2.7	0.1
Oseltamivir phosphate	1.00	1.0	—
Unspecified impurity	—	1.0	0.1
Total unspecified impurity	—	—	0.4

^a (3*R*,4*R*,5*S*)-4-Acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid.

^b 4-Acetylamino-3-hydroxybenzoic acid ethyl ester.

• PROCEDURE 2: OSELTAMIVIR RELATED COMPOUND A

Buffer: 1.54 g/L of ammonium acetate in water

Mobile phase: Acetonitrile, water, and *Buffer* (3:6:1)

Stock solution A: 50 µg/mL of USP Oseltamivir Related Compound A RS, prepared as follows: Dissolve in alcohol, using 5% of final volume, and dilute with water to volume.

Solution A: 1 µg/mL of USP Oseltamivir Related Compound A RS in water from *Stock solution A*

Standard solution: 10 mg/mL of USP Oseltamivir Phosphate RS in *Solution A*

Sample solution: 10 mg/mL of Oseltamivir Phosphate in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detectors: UV 210 nm and mass spectrometer

Column: 3.0-mm × 5-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 1 µL

Temperature: 40°

Use electrospray (+) ionization, a selected ion monitoring mode with m/z of 356.2 (protonated oseltamivir related compound A). Adjust the dwell time, fragmentation voltage, drying gas temperature, drying gas flow, nebulizer pressure, and capillary voltage as appropriate for an optimal response. [NOTE—A post-column flow splitter with a split ratio of about 3:1 is used.]

System suitability

Sample: *Standard solution*

[NOTE—The relative retention time for oseltamivir related compound A versus oseltamivir is about 2.6.]

Suitability requirements

Resolution: The oseltamivir related compound A peak (detected by MD-SIM mode) and the oseltamivir peak (detected by UV) are baseline resolved.

[NOTE—The resolution of the two components minimizes background noise and ion suppression effects for the trace of oseltamivir related compound A by the oseltamivir matrix.]

Relative standard deviation: NMT 15.0%, oseltamivir related compound A peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oseltamivir related compound A in the portion of Oseltamivir Phosphate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of oseltamivir related compound A from the *Sample solution*

- r_s = peak response of oseltamivir related compound A from the *Standard solution*
 C_s = concentration of USP Oseltamivir Related Compound A RS in the *Standard solution* (mg/mL)
 C_u = concentration of oseltamivir phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.01%

• **PROCEDURE 3: LIMIT OF TRIBUTYL PHOSPHINE OXIDE**

Blank: Transfer 1.0 mL of suitable derivatizing reagent¹ to a vial. Close the vial, shake, and heat for 20 min at 60°. Centrifuge the pyridinium salt precipitate.

Standard stock solution 1: 21 mg/mL of USP Tributyl Phosphine Oxide RS in pyridine

Standard stock solution 2: 21 mg/mL of USP Oseltamivir Phosphate RS in suitable derivatizing reagent. Close the vial, mix, and heat for 20 min at 60°. Centrifuge the pyridinium salt precipitate.

Standard solution: 21 µg/mL each of USP Tributyl Phosphine Oxide RS and USP Oseltamivir Phosphate RS in pyridine from *Standard stock solution 1* and *Standard stock solution 2*, respectively

Sample solution: Transfer 15 mg of Oseltamivir Phosphate to a vial. Add 1.0 mL of suitable derivatizing reagent. Close the vial, mix, and heat for 20 min at 60°. Centrifuge the pyridinium salt precipitate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 30-m capillary column coated with a 0.25-µm phase G1

Split ratio: 1:50

Split flow: 64 mL/min

Injection size: 1 µL

Temperature

Detector: 260°

Injection port: 260°

Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
180	0	180	2
180	8	250	10

Linear velocity: 27 cm/s

Carrier gas: Helium

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for tributyl phosphine oxide and oseltamivir phosphate are about 0.54 and 1.00, respectively.]

Suitability requirements

Relative standard deviation: NMT 10.0% for the tributyl phosphine oxide and oseltamivir phosphate peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tributyl phosphine oxide in the portion of Oseltamivir Phosphate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

¹ Tri-Sil Reagent (product number: 48999 0049001) may be obtained from Pierce: www.piercenet.com.

- r_u = peak response of tributyl phosphine oxide from the *Sample solution*
 r_s = peak response of tributyl phosphine oxide from the *Standard solution*
 C_s = concentration of tributyl phosphine oxide in the *Standard solution* (mg/mL)
 C_u = concentration of Oseltamivir Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

• **WATER DETERMINATION, Method I (921):** NMT 0.5%

• **OPTICAL ROTATION, Specific Rotation (781S)**

Sample solution: 10 mg/mL in water

Acceptance criteria: Between -30.7 and -32.6

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

• **USP REFERENCE STANDARDS (11)**

USP Oseltamivir Phosphate RS

USP Oseltamivir Related Compound A RS
 (3S,4R,5S)-Ethyl 4-acetamido-5-amino-2-azido-3-(pentan-3-yloxy)cyclohexanecarboxylate.

$C_{16}H_{29}N_5O_4$ 355.43

USP Tributyl Phosphine Oxide RS

$C_{12}H_{27}OP$ 218.32

Oseltamivir Phosphate Capsules

DEFINITION

Oseltamivir Phosphate Capsules contain Oseltamivir Phosphate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of oseltamivir ($C_{16}H_{28}N_2O_4$).

IDENTIFICATION

• The retention time of the major peaks of the *Sample solution* corresponds to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **OSELTAMIVIR**

Solution A: Dissolve 6.8 g of potassium dihydrogen phosphate in 980 mL of water. Adjust with 1 M potassium hydroxide solution to a pH of 6.0, and dilute with water to 1 L.

Mobile phase: Methanol, acetonitrile, and *Solution A* (245:135:620)

Diluent: Methanol, acetonitrile, and 0.01 N phosphoric acid (245:135:620)

Standard solution: 1 mg/mL of USP Oseltamivir Phosphate RS in *Diluent*

Sample solution: Weigh the contents of 20 Capsules, and mix. Prepare the equivalent of about 1 mg of oseltamivir phosphate per mL, based on the label claim, by first dispersing a suitable portion of the powder in about 40% of the flask volume of *Diluent* using an ultrasonic bath for about 20 min, and diluting with *Diluent* to volume. Centrifuge an aliquot of this solution, and use the supernatant.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 207 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 50°

Flow rate: 1.2 mL/min

Injection size: 15 µL

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of oseltamivir (C₁₆H₂₈N₂O₄) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Oseltamivir Phosphate RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of oseltamivir in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of oseltamivir, 312.40
 M_{r2} = molecular weight of oseltamivir phosphate, 410.40

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS**• DISSOLUTION (711)**

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 20 min

Detector: UV 240 nm

Standard solution: Prepare a solution in *Medium* having a known concentration of about 0.11 mg/mL of USP Oseltamivir Phosphate RS. Quantitatively dilute a portion of this solution with *Medium* to obtain a solution having a known concentration similar to the expected concentration in the solution under test.

Sample solution: Pass a portion of the solution under test through a suitable filter of 1-µm pore size.

Excipients solution: Suspend an amount of the placebo mixture equivalent to the weight of the excipients in one dosage unit and one empty Capsule shell in 900 mL of *Medium*. Heat to 37°, and filter.

AnalysisSamples: *Medium*, *Standard solution*, *Sample solution*, and *Excipients solution*

Determine the amount of oseltamivir phosphate (C₁₆H₂₈N₂O₄ · H₃PO₄) dissolved by measuring the absorbance at about 240 nm of the *Sample solution* and *Excipients solution* in comparison with the *Standard solution*, using the *Medium* as the blank. Calculate the percentage of oseltamivir phosphate dissolved:

$$\text{Result} = [(A_U - A_E) \times C_S \times V \times 100] / (A_S \times L)$$

- A_U = absorbance of the *Sample solution*
 A_E = absorbance of the *Excipients solution*
 C_S = concentration of USP Oseltamivir Phosphate RS in the *Standard solution*
 V = volume of *Medium*, 900 mL
 A_S = absorbance of the *Standard solution*
 L = label claim for oseltamivir phosphate (mg/Capsule)

Tolerances: NLT 75% (Q) of the labeled amount of oseltamivir phosphate is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements**IMPURITIES****• ORGANIC IMPURITIES**

Solution A, Mobile phase, Diluent, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

AnalysisSamples: *Standard solution* and *Sample solution*

Calculate the percentage of individual impurities in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response of each individual impurity from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Oseltamivir Phosphate RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of oseltamivir in the *Sample solution* (mg/mL)
 F = relative response factor from *Table 1*
 M_{r1} = molecular weight of oseltamivir, 312.40
 M_{r2} = molecular weight of oseltamivir phosphate, 410.40

Acceptance criteria: See *Table 1*.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity A ^a	0.18	1.4	2.0
Impurity B ^b	0.49	2.7	0.3
Oseltamivir phosphate	1.00	1.0	—
Impurity C ^c	1.45	0.9	0.5
Individual unidentified impurity	—	1.0	0.2
Total unidentified impurities	—	1.0	0.5
Total impurities	—	1.0	3.0

^a (3*R*,4*R*,5*S*)-4-Acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid.

^b 4-Acetylamino-3-hydroxybenzoic acid ethyl ester.

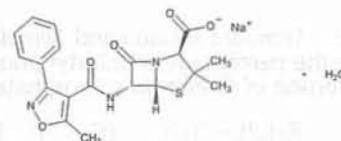
^c (3*R*,4*R*,5*S*)-4-Amino-5-acetylamino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid ethyl ester.

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in well-closed containers. Store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Oseltamivir Phosphate RS

Oxacillin Sodium

C₁₉H₁₈N₃NaO₅S · H₂O 441.43
 4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-6-[[[(5-methyl-3-phenyl-4-isoxazolyl)-carbonyl]amino]-7-oxo-, monosodium salt, monohydrate, [2*S*-(2*α*,5*α*,6*β*)]-;

Monosodium (2S,5R,6R)-3,3-dimethyl-6-(5-methyl-3-phenyl-4-isoxazolecarboxamido)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate [7240-38-2].

Anhydrous

$C_{19}H_{18}N_3NaO_5S$ 423.43
[1173-88-2].

DEFINITION

Oxacillin Sodium contains the equivalent of NLT 815 µg/mg and NMT 950 µg/mg of oxacillin ($C_{19}H_{19}N_3O_5S$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** (191), *Sodium*: Meets the requirements

ASSAY

PROCEDURE

Protect solutions containing oxacillin from light.

Solution A: 1.18 g/L of sodium 1-hexanesulfonate monohydrate and 0.8 mL/L of ammonium hydroxide in water, adjusted with phosphoric acid to a pH of 2.8–3.2

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20	30	70

Return to the original conditions and re-equilibrate the system.

Diluent: Acetonitrile and water (15:85)

System suitability stock solution: 0.1 mg/mL of USP Oxacillin Related Compound C RS in *Diluent*. Sonicate as needed to dissolve.

System suitability solution: 0.01 mg/mL of USP Oxacillin Related Compound C RS from *System suitability stock solution* and 1 mg/mL of USP Oxacillin Sodium RS in *Diluent*. Store this solution at 4°.

Standard solution: 1 mg/mL of USP Oxacillin Sodium RS in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

Sample solution: 1 mg/mL of Oxacillin Sodium in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Column: 40°

Autosampler: 4°

Flow rate: 1.5 mL/min

Injection volume: 2 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for oxacillin related compound C and oxacillin are about 0.96 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between oxacillin related compound C and oxacillin, *System suitability solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg/mg, of oxacillin ($C_{19}H_{19}N_3O_5S$) in the portion of Oxacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxacillin Sodium RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxacillin Sodium in the *Sample solution* (mg/mL)

P = potency of oxacillin in USP Oxacillin Sodium RS (µg/mg)

Acceptance criteria: 815–950 µg/mg

IMPURITIES

ORGANIC IMPURITIES

Protect solutions containing oxacillin from light.

Solution A: 6 g/L of anhydrous monobasic sodium phosphate, 0.56 g/L of sodium 1-hexanesulfonate monohydrate, and 0.05 g/L of edetate disodium. Adjust with phosphoric acid to a pH of 3.0–3.2.

Solution B: Acetonitrile

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3	90	10
30	50	50
40	15	85
45	15	85

Return to the original conditions and re-equilibrate the system.

Diluent: Acetonitrile and water (15:85)

System suitability stock solution: 0.1 mg/mL of USP Oxacillin Related Compound C RS in *Diluent*. Do not sonicate.

System suitability solution: 0.01 mg/mL of USP Oxacillin Related Compound C RS from *System suitability stock solution* and 1 mg/mL of USP Oxacillin Sodium RS in *Diluent*. Store this solution at 4°.

Standard solution: 0.01 mg/mL of USP Oxacillin Sodium RS in *Diluent*. Do not sonicate. Store this solution at 4°.

Sample solution: 1 mg/mL of Oxacillin Sodium in *Diluent*. Do not sonicate. Store this solution at 4°.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Column: 22°

Autosampler: 4°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between oxacillin related compound C and oxacillin, *System suitability solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 2.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Oxacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxacillin Sodium RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxacillin Sodium in the *Sample solution* (mg/mL)

P = potency of oxacillin in USP Oxacillin Sodium RS ($\mu\text{g}/\text{mg}$)

F_1 = conversion factor, 0.001 mg/ μg

F_2 = relative response factor (see Table 3)

Acceptance criteria: See Table 3. The reporting threshold is 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound A ^a	0.08	0.22	0.5
Oxacillin penicilloic acid ^{b,c}	0.66	0.40	1.5
	0.69		
Imidazothiazole analog ^d	0.68	1.0	0.5
Oxacillin penicilloic acid ^e	0.83	0.79	0.5
	0.84		
Thiooxacillin ^f	0.93	1.0	0.5
Oxacillin related compound C ^g	0.97	2.0	0.5
Oxacillin	1.0	—	—
Cloxacillin	1.09	1.0	1.0
Cloxacillin isomers ^h	1.17	1.0	0.5
N-(Penicillin-6-yl) oxacillinamide ⁱ	1.19	1.0	0.5
N-(Penicillin-6-yl) open ring oxacillinamide ^j	1.31	1.0	0.5

^a 6-Aminopenicillanic acid; (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^b (4S)-2-[Carboxy(5-methyl-3-phenylisoxazole-4-carboxamido)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^c The system resolves two isomers. The limit is for the sum of the isomers.

^d (3S,7R)-2,2-Dimethyl-5-(5-methyl-3-phenylisoxazol-4-yl)-2,3,7,7a-tetrahydroimidazo[5,1-b]thiazole-3,7-dicarboxylic acid.

^e (4S)-5,5-Dimethyl-2-[(5-methyl-3-phenylisoxazole-4-carboxamido)methyl]thiazolidine-4-carboxylic acid.

^f (2R,5R,6R)-3,3-Dimethyl-6-(5-methyl-3-phenylisoxazole-4-carboxamido)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^g Isoxazole carboxylic analog; 5-Methyl-3-phenylisoxazole-4-carboxylic acid.

^h (2S,5R,6R)-6-[3-(Chlorophenyl)-5-methylisoxazole-4-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

ⁱ (2S,5R,6R)-6-[(2S,5R,6R)-3,3-Dimethyl-6-(5-methyl-3-phenylisoxazole-4-carboxamido)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^j (2S,5R,6R)-6-[(R)-2-[(2R,4S)-4-Carboxy-5,5-dimethylthiazolidin-2-yl]-2-(5-methyl-3-phenylisoxazole-4-carboxamido)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Table 3 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	3.0

^a 6-Aminopenicillanic acid; (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^b (4S)-2-[Carboxy(5-methyl-3-phenylisoxazole-4-carboxamido)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^c The system resolves two isomers. The limit is for the sum of the isomers.

^d (3S,7R)-2,2-Dimethyl-5-(5-methyl-3-phenylisoxazol-4-yl)-2,3,7,7a-tetrahydroimidazo[5,1-b]thiazole-3,7-dicarboxylic acid.

^e (4S)-5,5-Dimethyl-2-[(5-methyl-3-phenylisoxazole-4-carboxamido)methyl]thiazolidine-4-carboxylic acid.

^f (2R,5R,6R)-3,3-Dimethyl-6-(5-methyl-3-phenylisoxazole-4-carboxamido)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^g Isoxazole carboxylic analog; 5-Methyl-3-phenylisoxazole-4-carboxylic acid.

^h (2S,5R,6R)-6-[3-(Chlorophenyl)-5-methylisoxazole-4-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

ⁱ (2S,5R,6R)-6-[(2S,5R,6R)-3,3-Dimethyl-6-(5-methyl-3-phenylisoxazole-4-carboxamido)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^j (2S,5R,6R)-6-[(R)-2-[(2R,4S)-4-Carboxy-5,5-dimethylthiazolidin-2-yl]-2-(5-methyl-3-phenylisoxazole-4-carboxamido)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

SPECIFIC TESTS

• **CRYSTALLINITY (695):** Meets the requirements

• **PH (791)**

Sample solution: 30 mg/mL

Acceptance criteria: 4.5–7.5

• **WATER DETERMINATION, Method I (921):** 3.5%–5.0%

• **STERILITY TESTS (71):** Where the label states that Oxacillin Sodium is sterile, it meets the requirements.

• **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Oxacillin Sodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.2 USP Endotoxin Units/mg of oxacillin.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, at controlled room temperature.

• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP REFERENCE STANDARDS (11)**

USP Endotoxin RS

USP Oxacillin Related Compound C RS

Isoxazole carboxylic analog;

5-Methyl-3-phenylisoxazole-4-carboxylic acid.

C₁₁H₉NO₃ 203.20

USP Oxacillin Sodium RS

Oxacillin Sodium Capsules**DEFINITION**

Oxacillin Sodium Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of oxacillin (C₁₉H₁₉N₃O₅S).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: 2.7 g/L of monobasic potassium phosphate
Mobile phase: Acetonitrile, methanol, and *Solution A* (300:100:700)

Standard solution: 0.11 mg/mL of USP Oxacillin Sodium RS in water. Use this solution on the day prepared.

Sample stock solution: Nominally 0.5 mg/mL of oxacillin in water, prepared as follows. Remove, as completely as possible, the contents of NLT 10 Capsules, and weigh. Mix, and transfer a suitable portion of the powder to a volumetric flask. Add water to volume, and mix for 10 min with the aid of a magnetic stirrer. Pass a portion of the solution through a suitable filter, discarding the first 5 mL of the filtrate.

Sample solution: Nominally 0.1 mg/mL of oxacillin from *Sample stock solution* in water. Use this solution on the day prepared.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4-mm × 30-cm; packing L11

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 1.6

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxacillin ($C_{19}H_{19}N_3O_5S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Oxacillin Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxacillin in the *Sample solution* (mg/mL)

P = potency of oxacillin in USP Oxacillin Sodium RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: USP Oxacillin Sodium RS in *Medium*

Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*, if necessary, to obtain a concentration that is similar to that of the *Standard solution*

Analysis: Determine the amount by a suitable validated spectrophotometric method.

Tolerances: NLT 75% (Q) of the labeled amount of oxacillin ($C_{19}H_{19}N_3O_5S$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements**SPECIFIC TESTS**• **WATER DETERMINATION** (921), *Method I*: NMT 6.0%**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight containers, at controlled room temperature.• **USP REFERENCE STANDARDS** (11)

USP Oxacillin Sodium RS

Oxacillin Injection**DEFINITION**

Oxacillin Injection is a sterile isoosmotic solution of Oxacillin Sodium in Water for Injection. It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of oxacillin ($C_{19}H_{19}N_3O_5S$). It contains dextrose as a tonicity-adjusting agent and one or more suitable buffer substances. It contains no preservatives.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: 2.7 g/L of monobasic potassium phosphate
Mobile phase: Acetonitrile, methanol, and *Solution A* (300:100:700)

Standard solution: 0.11 mg/mL of USP Oxacillin Sodium RS. Use this solution on the day prepared.

Sample solution: Nominally 0.1 mg/mL of oxacillin from one container of Injection that has been allowed to thaw. Use this solution on the day prepared.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4-mm × 30-cm; packing L11

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 1.6

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxacillin ($C_{19}H_{19}N_3O_5S$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxacillin Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxacillin in the *Sample solution* (mg/mL)

P = potency of oxacillin in USP Oxacillin Sodium RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

- **STERILITY TESTS** (71): It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.

- **pH** (791): 6.0–8.5

- **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections

- **PYROGEN TEST** (151): It meets the requirements, the test dose being a volume of undiluted Injection providing the equivalent of 20 mg/kg of oxacillin.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Maintain in the frozen state.
- **LABELING:** It meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just before use; it describes conditions for proper storage of the resultant solution; and it directs that the solution is not to be refrozen.
- **USP REFERENCE STANDARDS** (11)
USP Oxacillin Sodium RS

Oxacillin for Injection**DEFINITION**

Oxacillin for Injection contains an amount of Oxacillin Sodium equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of oxacillin ($C_{19}H_{19}N_3O_5S$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: 2.7 g/L of monobasic potassium phosphate
Mobile phase: Acetonitrile, methanol, and *Solution A* (300:100:700)

Standard solution: 0.11 mg/mL of USP Oxacillin Sodium RS. Use on the day prepared.

Sample solution 1 (where it is represented as being in a single-dose container): Nominally 0.1 mg/mL of oxacillin in water, prepared as follows. Constitute Oxacillin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents using a suitable hypodermic needle and syringe, and dilute with water to obtain a solution containing nominally 0.1 mg/mL of oxacillin. Use on the day prepared.

Sample solution 2 (where the label states the quantity of oxacillin in a given volume of constituted solution): Nominally 0.1 mg/mL of oxacillin in water, prepared as follows. Constitute Oxacillin for Injection with a volume of water corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution with water to obtain a solution containing nominally 0.1 mg/mL of oxacillin. Use on the day prepared.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4-mm × 30-cm; packing L11

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 1.6

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of the labeled amount of oxacillin ($C_{19}H_{19}N_3O_5S$) in the portion of Oxacillin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from *Sample solution 1* or *Sample solution 2*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxacillin Sodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxacillin in *Sample solution 1* or *Sample solution 2* (mg/mL)

P = potency of oxacillin in USP Oxacillin Sodium RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Where the test for *Uniformity of Dosage Units* has been performed, report the average of the determinations as the *Assay* value.

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements for *Content Uniformity* for individual containers using *Sample solution 1* or *Sample solution 2*, or both, as appropriate, prepared as directed in the *Assay*.

SPECIFIC TESTS

- **STERILITY TESTS** (71): It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.
- **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 0.2 USP Endotoxin Units/mg of oxacillin.
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** (1), *Specific Tests*, *Completeness and clarity of solutions*: Meets the requirements
- **WATER DETERMINATION** (921), *Method I*: NMT 6.0%
- **PH** (791)
Sample solution: Constitute as directed in the labeling.
Acceptance criteria: 6.0–8.5
- **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections

ADDITIONAL REQUIREMENTS**Change to read:**

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*, *Packaging for constitution* (CN 1-May-2017), at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
USP Endotoxin RS
USP Oxacillin Sodium RS

Oxacillin Sodium for Oral Solution**DEFINITION**

Oxacillin Sodium for Oral Solution contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of oxacillin ($C_{19}H_{19}N_3O_5S$). It contains one or more suitable buffers, colors, flavors, preservatives, and stabilizers.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Solution A: 2.7 g/L of monobasic potassium phosphate in water

Mobile phase: Acetonitrile, methanol, and *Solution A* (300:100:700)

Diluent: Acetonitrile and water (30:70)

Standard solution: 0.11 mg/mL of USP Oxacillin Sodium RS in *Diluent*. Use on the day prepared.

Sample stock solution: Constitute Oxacillin Sodium for Oral Solution as directed in the labeling and dilute with water to obtain a solution containing nominally 1 mg/mL of oxacillin.

Sample solution: Nominally 0.1 mg/mL of oxacillin from *Sample stock solution* in *Diluent*. Pass a portion of this solution through a filter of 0.5- μ m or finer pore size, discarding the first 2 mL of the filtrate. Use the clear filtrate. Use the *Sample solution* on the day prepared.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4-mm \times 30-cm; packing L11

Flow rate: 2 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 1.6

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxacillin ($C_{19}H_{19}N_3O_5S$) in the portion of Oxacillin Sodium for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- r_U = peak area from the *Sample solution*
 r_S = peak area from the *Standard solution*
 C_S = concentration of USP Oxacillin Sodium RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of oxacillin in the *Sample solution* (mg/mL)
 P = potency of oxacillin in USP Oxacillin Sodium RS (μ g/mg)
 F = conversion factor, 0.001 mg/ μ g
 Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• UNIFORMITY OF DOSAGE UNITS (905)

For solids packaged in single-unit containers

Acceptance criteria: Meets the requirements

• DELIVERABLE VOLUME (698): Meets the requirements

SPECIFIC TESTS

• WATER DETERMINATION (921), Method I: NMT 1.0%

• pH (791)

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 5.0–7.5

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in tight containers at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Oxacillin Sodium RS

DEFINITION

Oxaliplatin contains NLT 98.0% and NMT 102.0% of oxaliplatin ($C_8H_{14}N_2O_4Pt$), calculated on the dried basis.

[CAUTION—Great care should be taken in handling Oxaliplatin, because it is a potentially cytotoxic agent.]

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

• B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Polypropylene HPLC autosampler vials should be used.]

Buffer: Weigh 2.72 g of monobasic potassium phosphate (anhydrous) and 1.80 g of 1-pentanesulfonic acid sodium salt into a suitable container. Add 2000 mL of water, and mix well to completely dissolve all solids. Transfer 0.5 mL of triethylamine to the buffer solution, and mix thoroughly. Adjust the solution by dropwise addition of phosphoric acid to a pH of 4.30 ± 0.05 .

Mobile phase: Methanol and *Buffer* (3:17)

Oxaliplatin standard stock solution: 0.5 mg/mL of USP Oxaliplatin RS in water

Oxaliplatin related compound B standard stock solution: Transfer USP Oxaliplatin Related Compound B RS to a suitable volumetric flask, add 25% of the final volume of methanol, and sonicate for approximately 2 min to disperse the solids. Add approximately 65% of the final volume of 0.001 M nitric acid, and sonicate for an additional 30 min to dissolve the solids. Allow to cool if necessary. Dilute with 0.001 M nitric acid to volume, and mix to obtain a solution having a known concentration of 0.125 mg/mL. [NOTE—USP Oxaliplatin Related Compound B RS is converted to (SP-4-2)-diacqua[(1R, 2R)-cyclohexane-1,2-diamine-N,N']platinum during preparation of this solution.]

Oxaliplatin related compound C standard stock solution: 0.1 mg/mL of USP Oxaliplatin Related Compound C RS in water

System suitability solution: 2 mg/mL of Oxaliplatin in 0.005 M sodium hydroxide. Allow this solution to stand at room temperature for at least 5 days. Transfer 10 mL of this solution, 10 mL of *Oxaliplatin related compound B standard stock solution*, and 5 mL of *Oxaliplatin related compound C standard stock solution* into a 100-mL volumetric flask, and dilute with water to volume. [NOTE—The preparation of the *System suitability solution* forms diquodiaminocyclohexaneplatinum dimer.]

Standard solution: 0.1 mg/mL of USP Oxaliplatin RS in water from *Oxaliplatin standard stock solution*

Sample solution: 0.1 mg/mL of Oxaliplatin in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 50 μ L

System suitability

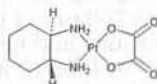
Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times, measured with respect to oxaliplatin, of oxaliplatin related compound C, oxaliplatin related compound B, and diquodiaminocyclohexaneplatinum dimer are 0.8, 2.7, and 6, respectively.]

Suitability requirements

Resolution: NLT 2.0 between oxaliplatin and oxaliplatin related compound C, *System suitability solution*

Oxaliplatin



$C_8H_{14}N_2O_4Pt$

397.29

[SP-4-2-(1R-trans)]-(1,2-Cyclohexanediamine-N,N')

[ethanedioato(2-)-O,O']platinum;

cis-[(1R,2R)-1,2-Cyclohexanediamine-N,N'] [oxalato(2-)-O,O']platinum [61825-94-3].

Tailing factor: Between 0.8 and 2.0 for oxaliplatin, *System suitability solution*

Relative standard deviation: NMT 2.0% for oxaliplatin, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxaliplatin ($C_8H_{14}N_2O_4Pt$) in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxaliplatin RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• LIMIT OF SILVER

Sample stock solution: Dissolve 100 mg of Oxaliplatin, weighed, in 50 mL of water to obtain a solution having a concentration of 2 mg/mL.

Sample solution: 1 mg/mL of Oxaliplatin in 0.5 M nitric acid from the *Sample stock solution*

Standard stock solution: Dilute a commercially available silver nitrate atomic absorption standard solution containing 1000 ppm of silver in 0.5 M nitric acid quantitatively, and stepwise if necessary, with 0.5 M nitric acid to obtain a 10-ppb solution.

Standard solution 1: Mix 20 μ L of the *Sample stock solution* and 8 μ L of the *Standard stock solution*, and dilute with 0.5 M nitric acid to 40 μ L.

Standard solution 2: Mix 20 μ L of the *Sample stock solution* and 16 μ L of the *Standard stock solution*, and dilute with 0.5 M nitric acid to 40 μ L.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometer equipped with a silver hollow-cathode lamp and graphite furnace

Analytical wavelength: Silver emission line of 328.1 nm

Blank: 0.5 M nitric acid

Analysis

Samples: *Sample solution*, *Standard solution 1*, and *Standard solution 2*

Plot the absorbances of the *Sample solution*, *Standard solution 1*, and *Standard solution 2* versus their concentrations, in ppb, of silver, and draw the straight line best fitting the three plotted points. The intercept on the x-axis of the extended regression line indicates the silver concentration in the *Sample solution*.

Calculate the concentration of silver, in ppm, in the portion of Oxaliplatin taken:

$$\text{Result} = (C/W) \times 100$$

C = absolute value of the intercept, in ppb of silver, on the x-axis

W = weight of Oxaliplatin taken for the preparation of the *Sample stock solution* (mg)

Acceptance criteria: NMT 5 ppm

Delete the following:

• HEAVY METALS

Standard stock solution: Transfer 1 mL each of 1000-ppm standard solutions of cadmium, chromium, copper, iron, nickel, and lead (commercially available) to a 100-mL volumetric flask. Add 5 mL of nitric acid, and dilute with water to volume.

Internal standard solution: Transfer 1 mL of a 10,000-ppm standard solution of yttrium (commercially available)

to a 100-mL volumetric flask, and dilute with 5% nitric acid to volume.

Standard solutions: Transfer 0.2, 2.0, and 20.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks. Add 1.0 mL of *Internal standard solution* and 5.0 mL of nitric acid to each flask, and dilute with water to volume. The concentrations of these solutions are 0.02, 0.20, and 2.00 ppm, respectively.

Blank solution: Transfer 1.0 mL of *Internal standard solution* and 5.0 mL of nitric acid to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: Weigh 1 g of Oxaliplatin into a 100-mL volumetric flask, and add 80 mL of water. Stir vigorously for several min with a magnetic stirrer until no more sample seems to be dissolving. Add 5 mL of nitric acid, and mix again until the sample is completely dissolved. Remove the stirrer bar from the flask, rinsing it before removal. Add 1.0 mL of the *Internal standard solution*, and dilute with water to volume.

Instrumental conditions

(See *Plasma Spectrochemistry* (730).)

Measure the responses of the elements cadmium, chromium, copper, iron, nickel, lead, and yttrium (internal standard), using an inductively coupled plasma-atomic optical emission spectrometer (ICP-OES), by measuring the emissions at 226.502, 283.563, 327.395, 259.940, 221.648, 220.353, and 371.029 nm, respectively. Optimize the instrument settings as directed by the manufacturer.

System suitability

Before samples are analyzed, the instrument must pass a suitable performance check. Generate the calibration curve, using the *Blank solution* and the *Standard solutions*, and run these solutions in the following order: the *Blank solution*, then the 0.02-, 0.20-, and 2.00-ppm *Standard solutions*. The linear regression coefficient is NLT 0.99; the response of the *Blank solution* is between –5.0 and 5.0 ppb for each element; and the responses of yttrium obtained from the *Standard solutions* are drifted by NMT 5.0% of the response obtained from the *Blank solution*. Run the 0.20-ppm *Standard solution*, and record the responses of each element: the relative standard deviations for replicate runs are NMT 5.0%; and the recovery against the calibration curve is between 95% and 105%. After samples are analyzed, the instrument must pass the same suitable performance check to ensure that the calibration is still valid.

Analysis

Sample: *Sample solution*

Record the responses of each element, and determine the concentration of each element, using the calibration graph. Calculate the content of total elements, in ppm, in the portion of Oxaliplatin taken:

$$\text{Result} = [(\Sigma C)/W] \times 100$$

C_i = concentration of each element in the *Sample solution* (ppm)

W = weight of Oxaliplatin taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 20 ppm • (Official 1-Jan-2018)

• CONTENT OF PLATINUM

Sample: Ignite an empty porcelain crucible fitted with a lid in a furnace at 800° for 30 min. Cool in a desiccator, and weigh. Add 200 mg of the Oxaliplatin, weighed, to the crucible, and ignite in a furnace by stepwise increments as follows. Introduce into the furnace, and increase the temperature to 200° within 15 min, then to 400° within 15 min, then to 600° within 15 min, then finally to 800° within 15 min. Allow to remain in the furnace at 800° for 30 min. Remove, cool in a desiccator, and reweigh.

Calculate the percentage of platinum in the portion of Oxaliplatin taken:

$$\text{Result} = (W_2/W_1) \times 100$$

W_2 = weight of residue after ignition (mg)

W_1 = weight of oxaliplatin before ignition (mg)

Acceptance criteria: 48.1%–50.1% of the oxaliplatin taken, on the dried basis

• **ORGANIC IMPURITIES, PROCEDURE 1: LIMIT OF OXALIC ACID**

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Polypropylene HPLC autosampler vials should be used.]

Buffer: Add 1.36 g of potassium dihydrogen phosphate to 10 mL of 10% tetrabutylammonium hydroxide in water, and dilute with water to 1000 mL. Adjust with phosphoric acid to a pH of 6.0.

Mobile phase: Acetonitrile and Buffer (1:4)

Standard stock solution: 0.06 mg/mL of USP Oxaliplatin Related Compound A RS in water

Standard solution: 15 µg/mL of USP Oxaliplatin Related Compound A RS in water from the *Standard stock solution*

System suitability solution: 0.05 mg/mL of sodium nitrate in water. Transfer 2 mL of this solution and 25 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with water to volume.

Sensitivity solution: 1.5 µg/mL of USP Oxaliplatin Related Compound A RS in water from the *Standard solution*

Sample solution: 2 mg/mL of Oxaliplatin in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

[NOTE—The elution order is sodium nitrate, followed by oxalic acid.]

Suitability requirements

Resolution: NLT 2.0 between oxalic acid and sodium nitrate, *System suitability solution*

Relative standard deviation: NMT 3.0% for oxalic acid, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxalic acid in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of oxalic acid from the *Sample solution*

r_S = peak response of oxalic acid from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of anhydrous oxalic acid, 90.03

M_{r2} = molecular weight of USP Oxaliplatin Related Compound A RS, 126.07

Acceptance criteria: NMT 0.1%

• **ORGANIC IMPURITIES, PROCEDURE 2: LIMIT OF (SP-4-2)-DIAQUA[(1R,2R)-CYCLOHEXANE-1,2-DIAMINE-N,N']PLATINUM, OXALIPLATIN RELATED COMPOUND C, AND UNSPECIFIED IMPURITIES**

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Polypropylene HPLC autosampler vials should be used.]

Mobile phase, Oxaliplatin standard stock solution, Oxaliplatin related compound B standard stock solution, Oxaliplatin related compound C standard stock solution, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.01 mg/mL of oxaliplatin, 0.01 mg/mL of oxaliplatin related compound B, and 0.004 mg/mL of oxaliplatin related compound C in water from *Oxaliplatin standard stock solution*, *Oxaliplatin related compound B standard stock solution*, and *Oxaliplatin related compound C standard stock solution*, respectively

Sample solution: 2 mg/mL of Oxaliplatin in water

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between oxaliplatin and oxaliplatin related compound C, *System suitability solution*

Tailing factor: Between 0.8 and 2.0 for oxaliplatin, *System suitability solution*

Relative standard deviation: NMT 3.0% for oxaliplatin, oxaliplatin related compound B, and oxaliplatin related compound C, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum from the *Sample solution*

r_S = peak response of (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound B RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum, 345.30

M_{r2} = molecular weight of USP Oxaliplatin Related Compound B RS, 433.28

[NOTE—USP Oxaliplatin Related Compound B RS is converted to (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum in solution preparation.]

Calculate the percentage of oxaliplatin related compound C in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxaliplatin related compound C from the *Sample solution*

r_S = peak response of oxaliplatin related compound C from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound C RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)

Calculate the percentage of diaquodiaminocyclohexaneplatinum dimer in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

- r_U = peak response of diaquodiaminocyclohexaneplatinum dimer from the *Sample solution*
 r_S = peak response of oxaliplatin related compound B from the *Standard solution*
 C_S = concentration of USP Oxaliplatin Related Compound B RS in the *Standard solution* (mg/mL)
 C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-*N,N'*]platinum, 345.30
 M_{r2} = molecular weight of USP Oxaliplatin Related Compound B RS, 433.28
 F = relative response factor for diaquodiaminocyclohexaneplatinum dimer, measured with respect to USP Oxaliplatin Related Compound B RS, 2.5

Calculate the percentage of any other unspecified impurity in the portion of Oxaliplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of any other unspecified impurity from the *Sample solution*
 r_S = peak response of oxaliplatin from the *Standard solution*
 C_S = concentration of oxaliplatin in the *Standard solution* (mg/mL)
 C_U = concentration of Oxaliplatin in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxaliplatin related compound C	0.8	—	0.1
Oxaliplatin	1.0	—	—
(SP-4-2)-Diaqua[(1R,2R)-cyclohexane-1,2-diamine- <i>N,N'</i>]platinum	2.7	—	0.1
Diaquodiaminocyclohexaneplatinum dimer	6	2.5	0.1
Any individual unspecified impurity	—	—	0.10
Total impurities ^a	—	—	0.30

^a Total impurities include oxalic acid (from Procedure 1) and all impurities from Procedure 2.

• ORGANIC IMPURITIES, PROCEDURE 3: LIMIT OF OXALIPLATIN RELATED COMPOUND D

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Polypropylene HPLC autosampler vials should be used.]

Mobile phase: Methanol and ethanol (7:3)

Oxaliplatin related compound D standard stock solution: 0.05 mg/mL of USP Oxaliplatin Related Compound D RS in methanol

Oxaliplatin related compound D standard solution: 15 µg/mL of USP Oxaliplatin Related Compound D RS in methanol from Oxaliplatin related compound D standard stock solution

Oxaliplatin standard stock solution: 0.75 mg/mL of USP Oxaliplatin RS in methanol

Oxaliplatin standard solution: 37.5 µg/mL of USP Oxaliplatin RS in methanol from Oxaliplatin standard stock solution

Oxaliplatin blank solution: Transfer 40 mL of Oxaliplatin standard stock solution to a 50-mL volumetric flask, and dilute with methanol to volume.

Standard solutions: Transfer 40 mL of Oxaliplatin standard stock solution to separate 50-mL volumetric flasks. Add 1.0, 3.0, and 5.0 mL of Oxaliplatin related compound D standard solution to each flask, and dilute with methanol to volume. The concentration of oxaliplatin in these solutions is 0.6 mg/mL. The concentrations of oxaliplatin related compound D in these solutions are 0.3, 0.9, and 1.5 µg/mL, respectively.

System suitability solution: Transfer 5.0 mL of Oxaliplatin standard solution and 4.0 mL of Oxaliplatin related compound D standard stock solution to a 50-mL volumetric flask, and dilute with methanol to volume.

Sample solution: Transfer 30 mg of Oxaliplatin into a 50-mL volumetric flask, and dilute with methanol to volume.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L70

Column temperature: 40°

Flow rate: 0.3 mL/min

Injection volume: 20 µL

Run time: 30 min

System suitability

Samples: 0.9-µg/mL Standard solution and System suitability solution

Suitability requirements

Resolution: NLT 1.5 between oxaliplatin and oxaliplatin related compound D, System suitability solution

Relative standard deviation: NMT 3.0% for the peak height ratio of oxaliplatin related compound D to the sum of oxaliplatin and oxaliplatin related compound D; 0.9-µg/mL Standard solution

Analysis

Samples: Standard solutions and Sample solution

Subtract the oxaliplatin related compound D peak height obtained in the Oxaliplatin blank solution from the oxaliplatin related compound D peak height obtained in the Standard solutions. [NOTE—USP Oxaliplatin RS may contain a small amount of oxaliplatin related compound D.] Plot a calibration curve for the Standard solutions with the peak height ratios of oxaliplatin related compound D to the sum of oxaliplatin and oxaliplatin related compound D on the y-axis and the concentration ratios of oxaliplatin related compound D, in µg/mL, to the sum of oxaliplatin and oxaliplatin related compound D concentrations, in mg/mL, on the x-axis. Read the concentration ratio of oxaliplatin related compound D, in µg/mL, to the sum of oxaliplatin and oxaliplatin related compound D, in mg/mL, in the Sample solution from the calibration curve.

Calculate the percentage of oxaliplatin related compound D in the portion of Oxaliplatin taken:

$$\text{Result} = R/10$$

- R = concentration ratio of oxaliplatin related compound D, in µg/mL, to the sum of oxaliplatin and oxaliplatin related compound D, in mg/mL, in the Sample solution from the calibration curve

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

• ACIDITY

Sample solution: Dissolve 100 mg in 50 mL of carbon dioxide-free water, and add 0.5 mL of phenolphthalein TS.

Acceptance criteria: The solution is colorless, and NMT 0.6 mL of 0.01 M sodium hydroxide is required to change the color to pink.

• BACTERIAL ENDOTOXINS TEST (85): NMT 1.0 USP Endotoxin Unit/mg of oxaliplatin

• LOSS ON DRYING (731)

Analysis: Dry 1 g at 100°–105° for 2 h.

Acceptance criteria: NMT 0.5%

• MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62): The total aerobic microbial count does not exceed 20 cfu/g, and the total combined molds and yeast count does not exceed 5 cfu/g.

• OPTICAL ROTATION, Specific Rotation (781S)

Sample solution: 5 mg/mL in water

Acceptance criteria: Between +74.5° and +78.0°, measured at 20°

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in tight containers, protected from light. Store at room temperature.

• USP REFERENCE STANDARDS (11)

USP Endotoxin RS

USP Oxaliplatin RS

USP Oxaliplatin Related Compound A RS

Oxalic acid dihydrate,

$C_2H_2O_4 \cdot 2H_2O$ 126.07

USP Oxaliplatin Related Compound B RS

[SP-4-2-(1*R*-trans)]-(1,2-Cyclohexanediamine-*N,N'*)dinitratoplatinum(II).

$C_6H_{14}N_4O_6Pt$ 433.28

USP Oxaliplatin Related Compound C RS

[1*R*-trans-(1,2-Cyclohexanediamine-*N,N'*)-trans-dihydroxido-[oxalato(2-)-*O,O'*]platinum(IV).

$C_8H_{16}N_2O_6Pt$ 431.30

USP Oxaliplatin Related Compound D RS

cis-[(1*S*,2*S*)-1,2-Cyclohexanediamine-*N,N'*][oxalato(2-)-*O,O'*]platinum.

$C_8H_{14}N_2O_4Pt$ 397.29

Oxaliplatin Injection

DEFINITION

Oxaliplatin Injection is a sterile solution of Oxaliplatin in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of oxaliplatin ($C_8H_{14}N_2O_4Pt$).

IDENTIFICATION

• A. ULTRAVIOLET ABSORPTION (197U)

Sample solution: 100 µg/mL

Medium: Water

• B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

[NOTE—All HPLC autosampler vials should be made of polypropylene.]

• PROCEDURE

Acidified water: Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Acidified water* (1:99)

System suitability solution: 0.1 mg/mL of USP Oxaliplatin RS and 0.1 mg/mL of USP Oxaliplatin System

Suitability RS in water. [NOTE—USP Oxaliplatin System Suitability RS is [SP-4-2-(1*R*-trans)]-(1,2-cyclohexanediamine-*N,N'*) dichloridoplatinum(II).]

Standard solution: 0.1 mg/mL of USP Oxaliplatin RS in water

Sample solution: 0.1 mg/mL of oxaliplatin in water, from the combined contents of NLT three vials of Injection

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for USP Oxaliplatin System Suitability RS and oxaliplatin are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between USP Oxaliplatin System Suitability RS and oxaliplatin

Tailing factor: NMT 2.0 for the oxaliplatin peak

Relative standard deviation: NMT 1.0% for the oxaliplatin peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxaliplatin ($C_8H_{14}N_2O_4Pt$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxaliplatin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• LIMIT OF OXALIC ACID

[NOTE—All HPLC autosampler vials should be made of polypropylene.]

Solution A: Dissolve 1.36 g of monobasic potassium phosphate in 10 mL of 10% tetrabutylammonium hydroxide, dilute with water to 1 L, and adjust with phosphoric acid to a pH of 6.0.

Mobile phase: Acetonitrile and *Solution A* (1:4)

Standard solution: 35 µg/mL of USP Oxaliplatin Related Compound A RS in water. [NOTE—USP Oxaliplatin Related Compound A RS is available as dihydrate oxalic acid.]

System suitability solution: 0.1 mg/mL of succinic acid in the *Standard solution*

Sensitivity solution: 3.5 µg/mL of USP Oxaliplatin Related Compound A RS in water from the *Standard solution*

Sample solution: Combined contents of NLT three vials of Injection

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

[NOTE—The relative retention times for succinic acid and oxalic acid are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between succinic acid and oxalic acid, *System suitability solution*

Tailing factor: 0.5–2.0 for the oxalic acid peak, *System suitability solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxalic acid in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of oxalic acid from the *Sample solution*

r_S = peak response of oxalic acid from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound A RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of anhydrous oxalic acid, 90.03

M_{r2} = molecular weight of oxaliplatin related compound A, 126.07

Acceptance criteria: NMT 0.6%

• LIMIT OF (SP-4-2)-DIAQUA[(1R,2R)-CYCLOHEXANE-1,2-DIAMINE-N,N']PLATINUM AND UNSPECIFIED IMPURITIES

[NOTE—All HPLC autosampler vials should be made of polypropylene.]

Solution A: Dissolve 1.36 g of monobasic potassium phosphate and 0.55 g of sodium heptanesulfonate in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Methanol and *Solution A* (19:81)

Solution C: Methanol and *Solution A* (50.5:49.5)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
45.0	0	100
45.5	100	0
53.0	100	0

System suitability solution: 2 mg/mL of USP Oxaliplatin RS in 0.005 M sodium hydroxide. Allow this solution to stand at room temperature for at least 5 days. Transfer 5 mL of this solution into a 50-mL volumetric flask, and dilute with water to volume. [NOTE—The preparation of the *System suitability solution* forms (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum and diaquodiaminocyclohexaneplatinum dimer.]

Standard stock solution: Transfer a weighed quantity of USP Oxaliplatin Related Compound B RS into a suitable volumetric flask, add a volume of methanol equivalent to about 25% of the final volume, and sonicate for approximately 2 min to disperse the solids. Add a volume of 0.01 M nitric acid equivalent to about 65% of the final volume, and sonicate for approximately 30 min to dissolve. Allow to cool if necessary, and dilute with 0.01 M nitric acid to volume to obtain a solution with a concentration of 0.125 mg/mL.

Standard solution: 31.25 µg/mL of USP Oxaliplatin Related Compound B RS in 0.01 M nitric acid, from the *Standard stock solution*. [NOTE—USP Oxaliplatin Related Compound B RS is converted to (SP-4-2)-diaqua[(1R,

2R)-cyclohexane-1,2-diamine-N,N']platinum in the *Standard solution* preparation.]

Sample solution: Combined contents of NLT three vials of Injection

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 7.5-cm; 3-µm packing L1

Column temperature: 10°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 8.0 between the peaks of (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum and diaquodiaminocyclohexaneplatinum dimer, *System suitability solution*

Tailing factor: NMT 2.0 for the (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum peak, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum, 345.30

M_{r2} = molecular weight of oxaliplatin related compound B, 433.28

F = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria

Individual impurities: See *Table 2*.

Total impurities: NMT 2.45%, from *Limit of Oxalic Acid* and *Limit of (SP-4-2)-Diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum and Unspecified Impurities*

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
(SP-4-2)-Diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum	1.0	1.0	0.65
Diaquodiaminocyclohexaneplatinum dimer ^a	1.4	2.5	0.50
Any individual unspecified impurity	—	4.0	0.2

^a (SP-4-2)-Di-µ-oxobis[(1R,2R)-cyclohexane-1,2-diamine-kN,kN']diplatinum.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 1.0 USP Endotoxin Unit/mg of oxaliplatin.

- **STERILITY TESTS** (71): It meets the requirements when tested as directed for *Membrane Filtration* in the *Test for Sterility of the Product to Be Examined*.
- **PH** (791): 4.0–7.0 using a polymer combination electrode
- **PARTICULATE MATTER IN INJECTIONS** (788): It meets the requirements for small-volume injections.
- **OTHER REQUIREMENTS**: It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature. Do not freeze.
- **LABELING**: Label it to indicate that it is to be diluted with a 5% dextrose solution. Oxaliplatin Injection must not be diluted in sodium chloride solutions or in chloride-containing solutions.
- **USP REFERENCE STANDARDS** (11)
 - USP Endotoxin RS
 - USP Oxaliplatin RS
 - USP Oxaliplatin Related Compound A RS
 - Oxalic acid dihydrate.
 - $C_2H_2O_4 \cdot 2H_2O$ 126.07
 - USP Oxaliplatin Related Compound B RS
 - [SP-4-2-(1*R-trans*)]-(1,2-Cyclohexanediamine-*N,N'*) dinitratoplatinum(II).
 - $C_6H_{14}N_4O_6Pt$ 433.28
 - USP Oxaliplatin System Suitability RS
 - [SP-4-2-(1*R-trans*)]-(1,2-Cyclohexanediamine-*N,N'*) dichloridoplatinum(II).
 - $C_6H_{14}Cl_2N_2Pt$ 380.17

Oxaliplatin for Injection

DEFINITION

Oxaliplatin for Injection is a sterile, lyophilized mixture of Oxaliplatin and Lactose Monohydrate. It contains NLT 90.0% and NMT 110.0% of the labeled amount of oxaliplatin ($C_8H_{14}N_2O_4Pt$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Use polypropylene HPLC autosampler vials.]

Acidified water: Adjust water with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and *Acidified water* (1:99)

System suitability solution: 0.1 mg/mL each of USP Oxaliplatin RS and USP Oxaliplatin System Suitability RS in water. [NOTE—USP Oxaliplatin System Suitability RS is compound [SP-4-2-(1*R-trans*)]-(1,2-cyclohexanediamine-*N,N'*) dichloridoplatinum(II).]

Standard solution: 0.1 mg/mL of USP Oxaliplatin RS in water

Sample solution: Constitute a suitable number of vials of Oxaliplatin for Injection with the appropriate amount of water to obtain a solution having a known concentration of about 0.1 mg/mL of oxaliplatin, based on the label claim.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection size: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for USP Oxaliplatin System Suitability RS and oxaliplatin are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the peaks of USP Oxaliplatin System Suitability RS and oxaliplatin, *System suitability solution*

Tailing factor: NMT 2.0 for the oxaliplatin peak, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxaliplatin ($C_8H_{14}N_2O_4Pt$) in the portion of Oxaliplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxaliplatin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

IMPURITIES

LIMIT OF OXALIC ACID

[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Use polypropylene HPLC autosampler vials.]

Buffer: Add 1.36 g of potassium dihydrogen phosphate to 10 mL of 10% tetrabutylammonium hydroxide in water, and dilute with water to 1000 mL. Adjust with phosphoric acid to a pH of 6.0.

Mobile phase: Acetonitrile and *Buffer* (1:4)

Standard stock solution: 0.06 mg/mL of USP Oxaliplatin Related Compound A RS in water. [NOTE—USP Oxaliplatin Related Compound A RS is available as oxalic acid dihydrate.]

Standard solution: 15 μg/mL of USP Oxaliplatin Related Compound A RS in water, from the *Standard stock solution*

System suitability stock solution: 0.05 mg/mL of sodium nitrate in water

System suitability solution: 1.0 μg/mL of sodium nitrate and 15 μg/mL of oxaliplatin related compound A in water, from the *System suitability stock solution* and *Standard stock solution*, respectively

Sensitivity solution: Make a 1-to-10 dilution of the *Standard solution* in water.

Sample solution: 2.0 mg/mL of oxaliplatin in water from Oxaliplatin for Injection

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 205 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Column temperature:** 40°**Flow rate:** 2 mL/min**Injection size:** 20 μL**System suitability****Samples:** *Standard solution*, *System suitability solution*, and *Sensitivity solution*

[NOTE—The relative retention times for sodium nitrate and oxalic acid are about 0.6 and 1.0, respectively.]

Suitability requirements**Resolution:** NLT 2.0 between oxalic acid and sodium nitrate, *System suitability solution***Relative standard deviation:** NMT 3.0% for the oxalic acid peak, *Standard solution***Sensitivity:** The signal-to-noise ratio of the peak at approximately the same retention time as that in the *Standard solution* is NLT 10, *Sensitivity solution*.**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of oxalic acid in the portion of Oxaliplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of oxalic acid from the *Sample solution* r_S = peak response of oxalic acid from the *Standard solution* C_S = concentration of USP Oxaliplatin Related Compound A RS in the *Standard solution* (mg/mL) C_U = concentration of oxaliplatin in the *Sample solution* (mg/mL) M_{r1} = molecular weight of anhydrous oxalic acid, 90.03 M_{r2} = molecular weight of USP Oxaliplatin Related Compound A RS, 126.07**Acceptance criteria:** NMT 0.5%**• LIMIT OF (SP-4-2)-DIAQUA[(1R,2R)-CYCLOHEXANE-1,2-DIAMINE-N,N']PLATINUM**[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Use polypropylene HPLC autosampler vials.]**Buffer:** Dissolve 1.36 g of potassium dihydrogen phosphate and 1 g of sodium 1-heptanesulfonate in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.**Mobile phase:** Acetonitrile and *Buffer* (1:4)**System suitability solution:** 2 mg/mL of USP Oxaliplatin RS in 0.005 M sodium hydroxide. Allow this solution to stand at room temperature for at least 5 days.[NOTE—Sonicate if necessary.] Transfer 5 mL of this solution into a 50-mL volumetric flask, and dilute with water to volume. [NOTE—The preparation of the *System suitability solution* forms diaquodiaminocyclohexaneplatinum dimer and (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum.]**Standard solution:** Transfer USP Oxaliplatin Related Compound B RS to a suitable volumetric flask, add 25% of the final volume of methanol, and sonicate for approximately 30 min to dissolve. Allow to cool if necessary, and dilute with water to volume to obtain a solution having a known concentration of about 0.0125 mg/mL. [NOTE—When preparing the solution, USP Oxaliplatin Related Compound B RS is converted to (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum.]**Sample solution:** Use the *Sample solution* from the test for *Limit of Oxalic Acid*.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 215 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Column temperature:** 40°**Flow rate:** 2 mL/min**Injection size:** 20 μL**System suitability****Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum and diaquodiaminocyclohexaneplatinum dimer are about 1.0 and 1.5, respectively.]

Suitability requirements**Resolution:** NLT 2.0 between (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum and diaquodiaminocyclohexaneplatinum dimer, *System suitability solution***Relative standard deviation:** NMT 3.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum in the portion of Oxaliplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum from the *Sample solution* r_S = peak response of (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum from the *Standard solution* C_S = concentration of USP Oxaliplatin Related Compound B RS in the *Standard solution* (mg/mL) C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL) M_{r1} = molecular weight of (SP-4-2)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-N,N']platinum, 345.30 M_{r2} = molecular weight of USP Oxaliplatin Related Compound B RS, 433.28**Acceptance criteria:** NMT 0.5%**• LIMIT OF RELATED COMPOUND C AND UNSPECIFIED IMPURITIES**[NOTE—Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the *Sample solution* within 20 min of preparation. Use polypropylene HPLC autosampler vials.]**Mobile phase:** Proceed as directed in the *Assay*.**Standard stock solution:** 0.1 mg/mL each of USP Oxaliplatin RS and USP Oxaliplatin Related Compound C RS in water**Standard solution:** 0.01 mg/mL each of USP Oxaliplatin RS and USP Oxaliplatin Related Compound C RS in water, from the *Standard stock solution***System suitability stock solution:** Dissolve USP Oxaliplatin System Suitability RS in methanol, and sonicate for approximately 10 min to obtain a solution having a concentration of 0.1 mg/mL.**System suitability solution:** Transfer 10 mL each of the *Standard stock solution* and the *System suitability stock solution* into a 100-mL volumetric flask, and dilute with water to volume.**Sample solution:** Use the *Sample solution* from the test for *Limit of Oxalic Acid*.**Chromatographic system:** Proceed as directed in the *Assay*, except for the injection size.**Injection size:** 10 μL**System suitability****Samples:** *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between [SP-4-2-(1*R-trans*)]-(1,2-cyclohexanediamine-*N,N'*) dichloridoplatinum(II) and oxaliplatin, *System suitability solution*

Tailing factor: NMT 2.0 for the oxaliplatin peak, *System suitability solution*

Relative standard deviation: NMT 3.0% each for the oxaliplatin and oxaliplatin related compound C peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of oxaliplatin related compound C in the portion of Oxaliplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxaliplatin related compound C from the *Sample solution*

r_S = peak response of oxaliplatin related compound C from the *Standard solution*

C_S = concentration of USP Oxaliplatin Related Compound C RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Oxaliplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of oxaliplatin from the *Standard solution*

C_S = concentration of USP Oxaliplatin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxaliplatin in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxaliplatin related compound C ^a	0.6	0.3
[SP-4-2-(1 <i>R-trans</i>)]-(1,2-Cyclohexanediamine- <i>N,N'</i>) dichloridoplatinum(II) ^b	0.8	—
Oxaliplatin	1.0	—
Any individual unspecified impurity	—	0.2
Total impurities ^c	—	1.5

^a [1*R-trans*-(1,2-Cyclohexanediamine-*N,N'*)]-*trans*-dihydroxido-[oxalato(2-)-O,O']platinum(IV).

^b The relative retention time of [SP-4-2-(1*R-trans*)]-(1,2-cyclohexanediamine-*N,N'*) dichloridoplatinum(II) has been included for system suitability purposes only.

^c Includes oxalic acid, (SP-4-2)-diaqua[(1*R,2R*)-cyclohexane-1,2-diamine-*N,N'*]platinum, oxaliplatin related compound C, and the total of the individual unspecified impurities.

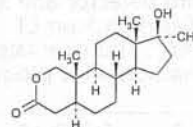
SPECIFIC TESTS

- PH (791):** 4.0–7.0 using a polymer combination electrode, determined in a solution constituted as directed in the labeling
- PARTICULATE MATTER IN INJECTIONS (788):** It meets the requirements for small-volume injections.
- CONSTITUTED SOLUTION:** At the time of use, it meets the requirements under *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

- BACTERIAL ENDOTOXINS TEST (85):** NMT 1.0 USP Endotoxin Unit/mg of oxaliplatin
- STERILITY TESTS (71):** Meets the requirements
- WATER DETERMINATION, Method 1 (921):** NMT 4.0%
- OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS**Change to read:**

- PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017). Store at controlled room temperature.
- LABELING:** Label it to indicate that it is to be diluted with a suitable parenteral vehicle before intravenous infusion.
- USP REFERENCE STANDARDS (11)**
 - USP Endotoxin RS
 - USP Oxaliplatin RS
 - [SP-4-2-(1*R-trans*)]-(1,2-Cyclohexanediamine-*N,N'*) [ethanedioato(2-)-O,O']platinum.
C₈H₁₄N₂O₄Pt 397.29
 - USP Oxaliplatin Related Compound A RS
 - Oxalic acid dihydrate.
C₂H₂O₄ · 2H₂O 126.07
 - USP Oxaliplatin Related Compound B RS
 - [SP-4-2-(1*R-trans*)]-(1,2-Cyclohexanediamine-*N,N'*) dinitratoplatinum(II).
C₆H₁₄N₄O₆Pt 433.28
 - USP Oxaliplatin Related Compound C RS
 - [1*R-trans*-(1,2-Cyclohexanediamine-*N,N'*)]-*trans*-dihydroxido-[oxalato(2-)-O,O']platinum(IV).
C₈H₁₆N₂O₆Pt 431.30
 - USP Oxaliplatin System Suitability RS
 - [SP-4-2-(1*R-trans*)]-(1,2-Cyclohexanediamine-*N,N'*) dichloridoplatinum (II).
C₆H₁₄Cl₂N₂Pt 380.17

Oxandrolone

C₁₉H₃₀O₃ 306.44

2-Oxaandrostan-3-one, 17-hydroxy-17-methyl-, (5α,17β)-17β-Hydroxy-17-methyl-2-oxa-5α-androstan-3-one [53-39-4].

» Oxandrolone contains not less than 98.0 percent and not more than 102.0 percent of C₁₉H₃₀O₃, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards (11)—

- USP Oxandrolone RS
- USP Oxandrolone Related Compound A RS (7,8-Didehydro-oxandrolone) or (17β-hydroxy-17α-methyl-2-oxa-5α-androst-7-en-3-one).
- USP Oxandrolone Related Compound B RS (4-Oxa-isomer) or (17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one).
- USP Oxandrolone Related Compound C RS Anhydro-oxandrolone or (17,17-dimethyl-18-nor-2-oxa-5α-androst-13-en-3-one).

Identification—

A: Infrared Absorption (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Specific rotation (781S): between -18° and -24° .

Test solution: 10 mg per mL, in chloroform.

Loss on drying (731)—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.2%.

Related compounds—

Solution A: acetonitrile.

Solution B: water.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Blank solution—Prepare a mixture of *Solution A* and *Solution B* (50:50).

Standard stock solution—Dissolve accurately weighed quantities of USP Oxandrolone Related Compound A RS, USP Oxandrolone Related Compound B RS, USP Oxandrolone Related Compound C RS, and USP Oxandrolone RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having known concentrations of about 4 μg of USP Oxandrolone Related Compound A RS per mL, 120 μg of USP Oxandrolone Related Compound B RS per mL, 4 μg of USP Oxandrolone Related Compound C RS per mL, and 200 μg of USP Oxandrolone RS per mL. [NOTE—Sonicate if necessary to dissolve.]

Standard solution—Dilute 1.0 mL of the *Standard stock solution* with 4.0 mL of acetonitrile and 5.0 mL of water, and mix.

Test solution—Weigh accurately 40 mg of Oxandrolone into a 10-mL volumetric flask, dissolve in 5.0 mL of acetonitrile using an ultrasonic bath, dilute with water to volume, and mix. [NOTE—The *Test solution*, the *Standard solution*, and the *Blank solution* are made up fresh and injected immediately.]

Chromatographic system—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm \times 25-cm column that contains 5- μm packing L1. The column temperature is maintained at 40° . The flow rate is about 0.7 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	50	50	equilibration
0–30	50→100	50→0	linear gradient
30–32	100→50	0→50	linear gradient
32–40	50	50	re-equilibration

Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution, R , between oxandrolone related compound A and oxandrolone related compound B is not less than 1.5, and the resolution, R , between oxandrolone related compound B and oxandrolone is not less than 2.0; the tailing factor is not more

than 1.5; and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 50 μL) of the *Blank solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of oxandrolone related compound A in the portion of Oxandrolone taken by the formula:

$$(C/W)(r_U / r_S)$$

in which C is the concentration, in μg per mL, of oxandrolone related compound A in the *Standard solution*; W is the weight, in mg, of Oxandrolone taken to prepare the *Test solution*; r_U is the peak area of oxandrolone related compound A in the chromatogram of the *Test solution*; and r_S is the peak area obtained for oxandrolone related compound A in the chromatogram of the *Standard solution*.

Calculate the percentage of oxandrolone related compound C, methyltestosterone, $\Delta 1$ -mestalone, specified unknown impurity 1, and each impurity eluting at a relative retention time greater than or equal to 2.2 (relative to retention time of oxandrolone) by the formula:

$$(1/F)(C/W)(r_U / r_S)$$

in which F is the relative response factor (see accompanying table for values); C is the concentration, in μg per mL, of oxandrolone related compound C in the *Standard solution*; W is the weight, in mg, of Oxandrolone taken to prepare the *Test solution*; r_U is the peak area of oxandrolone related compound C, methyltestosterone, $\Delta 1$ -mestalone, specified unknown impurity 1, or each impurity eluting at a relative retention time greater than or equal to 2.2 in the chromatogram of the *Test solution*; and r_S is the peak area obtained for oxandrolone related compound C in the chromatogram of the *Standard solution*.

Calculate the percentage of each impurity, except oxandrolone related compound A, oxandrolone related compound C, methyltestosterone, $\Delta 1$ -mestalone, specified unknown impurity 1, and other impurities eluting at relative retention times greater than or equal to 2.2 by the formula:

$$(1/F)(C/W)(r_U / r_S)$$

in which F is the relative response factor for each impurity (see accompanying table for values); C is the concentration, in μg per mL, of USP Oxandrolone RS in the *Standard solution*; W is the weight, in mg, of Oxandrolone taken to prepare the *Test solution*; r_U is the peak area of each impurity, in the chromatogram of the *Test solution*, other than peak areas of oxandrolone related compound A, oxandrolone related compound C, methyltestosterone, $\Delta 1$ -mestalone, specified unknown impurity 1, and other impurities eluting at relative retention times greater than or equal to 2.2; and r_S is the peak area obtained for oxandrolone in *Standard solution*. Disregard any peak observed in the chromatogram obtained from the *Blank solution*. Disregard any impurity peak that is less than 0.05%. The impurities meet the requirements specified in the accompanying table.

Compound	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Secodicarboxylic acid ¹	0.46	4.1	0.1
7,8-Didehydro-oxandrolone ² (Oxandrolone related compound A)	0.90	—	0.1
4-Oxa-isomer ³ (Oxandrolone related compound B)	0.94	1.4	0.3
Oxandrolone	1.00	—	—
Oxandrolone open lactone methyl ester ⁴	1.09	1.5	0.1
Secoacid anhydride ⁵	1.12	2.5	0.1
Methyltestosterone ⁶	1.25	0.8 ^a	0.1
17-epi-Oxandrolone ⁷	1.33	1.0	0.3
$\Delta 1$ -Mestalone ⁸	1.48	1.3 ^a	0.1
4-Oxa-isomer (beta epimer) ⁹	1.52	1.4	0.3
Specified unknown impurity 1	1.63	0.6 ^a	0.1
Oxandrolone-17-acetate ¹⁰	2.14	1.9	0.1
Anhydro-oxandrolone ¹¹ (Oxandrolone related compound C)	3.29	—	0.5
Individual unknown impurity	—	1.0	0.1
Total impurities	—	—	1.0

¹ 17 β -Hydroxy-17 α -methyl-2-nor-5 α -androstan-1,3-dioic acid.

² 17 β -Hydroxy-17 α -methyl-2-oxa-5 α -andro-7-en-3-one.

³ 17 β -Hydroxy-17 α -methyl-4-oxa-5 α -androstan-3-one.

⁴ Methyl-(1,17 β -dihydroxy-17 α -methyl-1,3-seco-2-nor-5 α -androstan-3-one.

⁵ 17 β -Hydroxy-17 α -methyl-2-oxa-5 α -androstan-1,3-dione.

⁶ 17 β -Hydroxy-17 α -methyl-5 α -andro-4-ene-3-one.

⁷ 17 α -Hydroxy-17 β -methyl-2-oxa-5 α -androstan-3-one.

⁸ 17 β -Hydroxy-17 α -methyl-5 α -andro-1-ene-3-one.

⁹ 17 β -Hydroxy-17 α -methyl-4-oxa-5 β -androstan-3-one.

¹⁰ 17 β -Hydroxy-17 α -methyl-2-oxa-5 α -androstan-3-one 17-acetate.

¹¹ 17,17-Dimethyl-18-nor-2-oxa-5 α -andro-13-en-3-one.

^a F values relative to oxandrolone related compound C.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Oxandrolone RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 3 mg per mL. [NOTE—Sonicate if necessary to dissolve.]

Assay preparation—Transfer to a suitable volumetric flask an accurately weighed quantity of Oxandrolone, and dissolve in and dilute with acetonitrile to volume to obtain a solution having a concentration of about 3 mg per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm \times 25-cm column that contains packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of C₁₉H₃₀O₃ in the portion of Oxandrolone taken by the formula:

$$VC(r_u / r_s)$$

in which V is the final volume, in mL, of the *Assay preparation*; C is the concentration, in mg per mL, of USP Oxandrolone RS in the *Standard preparation*; and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Oxandrolone Tablets

» Oxandrolone Tablets contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of oxandrolone (C₁₉H₃₀O₃).

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—When more than one *Dissolution Test* is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.

USP Reference standards (11)—

USP Oxandrolone RS

Identification—

A: Thin-Layer Chromatographic Identification Test (201)—

Standard solution: 5 mg per mL in chloroform.

Test solution—Transfer a portion of finely powdered Tablets, equivalent to about 20 mg of oxandrolone, to a 50-mL stoppered centrifuge tube, add 4 mL of chloroform, shake by mechanical means for 10 minutes, centrifuge for about 15 minutes, and filter a portion of the chloroform layer.

Application volume: 10 μ L.

Developing solvent system: a mixture of chloroform and methanol (19:1).

Procedure—Locate the spots on the plate by lightly spraying with dilute sulfuric acid (1 in 2) and heating on a hot plate or under a lamp until spots appear: the R_f value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Standard solution*.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Change to read:

Dissolution (711)—

TEST 1—

Medium: a solution of water and isopropanol (7:3); 500 mL.

Apparatus 2: 100 rpm.

Time: 60 minutes.

Determine the amount of C₁₉H₃₀O₃ dissolved by employing the following method.

Internal standard solution—Dissolve accurately weighed quantities of 17 α -methyltestosterone, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a concentration of about 0.2 mg per mL (for Tablets with a 2.5-mg label claim) and about 0.8 mg per mL (for Tablets with a 10-mg label claim).

Standard solution—Dissolve an accurately weighed quantity of USP Oxandrolone RS, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a concentration of about 1 mg per mL.

Working standard solution—For Tablets labeled to contain 2.5 mg: combine 100 μ L of the *Standard solution*, 400 μ L of the *Internal standard solution*, and 1500 μ L of acetonitrile. For Tablets labeled to contain 10 mg: combine 100 μ L of the *Standard solution*, 100 μ L of the *Internal standard solution*, and 1800 μ L of acetonitrile.

Test solution—Withdraw 25 mL of the solution under test from the vessel. Pass through a 0.45- μ m polytetrafluoroethylene filter. Transfer 20 mL of the filtrate to a separatory funnel, add 400 μ L of the *Internal standard solution*, 40 mL of a 10% potassium chloride solution, and 8 mL of chloroform. In separate separatory funnels, prepare an extraction blank and an internal standard blank in a similar manner using 20 mL of filtered *Medium* in place of the solution under test and excluding the *Internal standard solution* from the extraction blank. Shake each funnel, and allow the layers to separate. Collect the lower chloroform layer. Repeat the extraction procedure one more time. Evaporate the solvents under a stream of nitrogen at 45° until just dry. Reconstitute the dried residue with 2 mL of acetonitrile (for Tablets with a 2.5-mg label claim) or with 8 mL of acetonitrile (for Tablets with a 10-mg label claim), and sonicate for 10 minutes.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm \times 30-m column coated with a 0.5- μ m phase G27. The carrier gas is helium, flowing at a rate of about 16.8 mL per minute. The injection port and detector temperatures are maintained at 190° and 320°, respectively. The chromatograph is programmed as follows. The column temperature is initially 180°. Upon injection, the column temperature is increased at a rate of 25° per minute to 280°, and maintained at 280° for 3 minutes. Then the column temperature is increased at a rate of 10° per minute to 320°, and maintained at 320° for 3 minutes. Chromatograph the acetonitrile, the extraction blank, and the internal standard blank, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5. Make two injections of the *Working standard solution*, and record the peak responses. The average oxandrolone/*Internal standard solution* peak area percent comparison is between 98.0% and 102.0%. The resolution, *R*, between the oxandrolone peak and the nearest eluting peak is equal to or greater than 1.5.

Procedure—Separately inject equal volumes (0.5 μ L) of the *Working standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{19}H_{30}O_3$ released by the formula:

$$\frac{C_s \times \text{sample ratio} \times V_{UF} \times 500 \times 100}{\text{Standard ratio} \times V_{UI} \times LC}$$

in which C_s is the concentration, in mg per mL, of oxandrolone in the *Standard solution*; sample ratio is the area ratio of oxandrolone to 17 α -methyltestosterone in the sample injection for each *Test solution*; V_{UF} is the final volume, in mL, of the sample after reconstitution of the dry residue; 500 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; standard ratio is the mean area ratio of oxandrolone to 17 α -methyltestosterone in all injections of the *Standard solution*; V_{UI} is the initial sample volume, in mL, used in the extraction; and *LC* is the Tablet label claim, in mg.

Tolerances—Not less than 75% (*Q*) of the labeled amount of oxandrolone ($C_{19}H_{30}O_3$) is dissolved in 60 minutes.

TEST 2—If the product complies with this test, the labeling indicates that it meets *USP Dissolution Test 2*.

Medium: 1% polysorbate 80 in water; 500 mL, deaerated.

Apparatus 2: 100 rpm.

Time: 120 minutes.

Determine the amount of $C_{19}H_{30}O_3$ dissolved by employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard stock solution—Transfer about 20 mg of USP Oxandrolone RS, accurately weighed, to a 200-mL volumetric flask. Add about 20 mL of acetonitrile, and sonicate to dissolve. Dilute with *Medium* to volume, and mix.

Working standard solution—Quantitatively dilute the *Standard stock solution* with *Medium* to obtain a solution having a final concentration of about 5 μ g per mL for Tablets with a label claim of 2.5 mg, or a final concentration of about 20 μ g per mL for Tablets with a label claim of 10 mg.

Test solution—Withdraw about 10 mL of the solution under test from the vessel. Centrifuge in a glass tube at 2000 rpm for 10 minutes.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a refractive index detector and a 4.6-mm \times 25-cm column that contains 5- μ m packing L1. The column is maintained at 30°, and the detector is maintained at 50°. The flow rate is about 1.5 mL per minute. Chromatograph the *Working standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; the column efficiency is not less than 4000 theoretical plates; and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 100 μ L) of the *Working standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{19}H_{30}O_3$ released by the formula:

$$\frac{r_U \times C_s \times D \times 500 \times 100}{r_s \times LC}$$

in which r_U and r_s are the peak responses obtained from the *Test solution* and *Working standard solution*, respectively; C_s is the concentration, in mg per mL, of the *Working standard solution*; *D* is the dilution factor of the *Test solution*; 500 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and *LC* is the Tablet label claim, in mg.

Tolerances—Not less than 65% (*Q*) of the labeled amount of $C_{19}H_{30}O_3$ is dissolved in 120 minutes.

TEST 3—If the product complies with this test, the labeling indicates that it meets *USP Dissolution Test 3*.

Medium: 0.1 N hydrochloric acid containing 0.75% of sodium lauryl sulfate; 500 mL for Tablets labeled to contain 2.5 mg, 900 mL for Tablets labeled to contain 10 mg, deaerated with helium.

Apparatus 2: 75 rpm.

Time: 90 minutes.

Determine the amount of $C_{19}H_{30}O_3$ released by employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard stock solution—Transfer about 20 mg, accurately weighed, of USP Oxandrolone RS to a 100-mL volumetric flask. Dissolve in approximately 5 mL of acetonitrile, and sonicate for 10 minutes. Dilute with *Medium* to volume, and mix.

Working standard solution—Transfer 8.0 mL of the *Standard stock solution* to a 200-mL volumetric flask, dilute with *Medium* to volume, and mix.

Test solution—Pass the solution under test through a suitable filter having a porosity of 0.45 μ m.

Chromatographic system—The liquid chromatograph is equipped with a refractive index detector and a 4.6-mm × 3-cm column (ERR 1-Jun-2016) that contains 5-μm packing L1. The flow rate is about 1.0 mL per minute. The temperatures of the detector and the column are both maintained at 35°. Chromatograph the *Working standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 200 μL) of the *Working standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of C₁₉H₃₀O₃ dissolved by the formula:

$$\frac{r_U \times C_S \times 500 \times 100}{r_S \times LC}$$

in which r_U and r_S are the peak responses for the *Test solution* and the *Working standard solution*, respectively; C_S is the concentration, in mg per mL, of oxandrolone in the *Working standard solution*; 500 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and LC is the Tablet label claim, in mg.

Tolerances—Not less than 75% (Q) of the labeled amount of C₁₉H₃₀O₃ is dissolved in 90 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (62:38). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Diluent—Prepare a mixture of water and acetonitrile (1:1).

Standard preparation—Dissolve an accurately weighed quantity of USP Oxandrolone RS in *Diluent*, and dilute with *Diluent* to obtain a solution having a known concentration of about 0.5 mg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 50 mg of oxandrolone, to a 100-mL volumetric flask. Add 50 mL of *Diluent*, sonicate for 30 minutes with frequent shaking, and shake for an additional 15 minutes using a mechanical shaker. Dilute with *Diluent* to volume, and centrifuge. Use the supernatant as the *Assay preparation*.

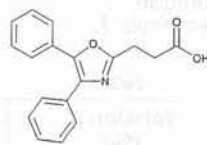
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a refractive index detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The column is maintained at 30°, and the detector is maintained at 50°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; the column efficiency is not less than 5000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the oxandrolone peaks. Calculate the quantity, in percent of label claim, of oxandrolone (C₁₉H₃₀O₃) in the portion of Tablets taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S is the concentration, in mg per mL, of oxandrolone in the *Standard preparation*; C_U is the concentration, in mg per mL, of oxandrolone in the *Assay preparation*, based on the label claim; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Oxaprozin



C₁₈H₁₅NO₃ 293.32
2-Oxazolepropanoic acid, 4,5-diphenyl-;
4,5-Diphenyl-2-oxazolepropionic acid [21256-18-8].

DEFINITION

Oxaprozin contains NLT 98.0% and NMT 102.0% of oxaprozin (C₁₈H₁₅NO₃), calculated on the dried basis.

[NOTE—Because of light sensitivity, protect all oxaprozin samples and standard solutions from light.]

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K): Previously dried at 105° for 2 h
- **B.** The retention time of the oxaprozin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: 0.1% phosphoric acid, pH 2.0 ± 0.1

Mobile phase: *Solution A* and acetonitrile (550:450)

Standard solution: 0.2 mg/mL of USP Oxaprozin RS in acetonitrile

Sample solution: 0.2 mg/mL of Oxaprozin in acetonitrile

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 25°

Flow rate: 1.0 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxaprozin (C₁₈H₁₅NO₃), in the portion of Oxaprozin taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

r_U = peak response of oxaprozin from the *Sample solution*

r_S = peak response of oxaprozin from the *Standard solution*

C_S = concentration of USP Oxaprozin RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxaprozin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.3%
- **ARSENIC, Method II** (211): NMT 1 ppm

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1-Jan-2018)

• **ORGANIC IMPURITIES**

Solution A: 0.1% phosphoric acid adjusted with phosphoric acid to a pH of 2.00 ± 0.1

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
20	70	30
21	70	30
60	0	100
61	70	30
70	70	30

Diluent A: Acetonitrile, methylene chloride, and water (48:1:1)

Diluent B: Acetonitrile and water (1:1)

Standard stock solution: 200 µg/mL of USP Oxaprozin RS in acetonitrile

Standard solution: 5 µg/mL of USP Oxaprozin RS in Diluent A from the Standard stock solution

System suitability stock solution: 200 µg/mL of benzil in acetonitrile

System suitability solution: 10 µg/mL each of benzil and USP Oxaprozin RS in Diluent A from the System suitability stock solution and Standard stock solution, respectively

Sample solution A: 1 mg/mL of Oxaprozin prepared as follows. Transfer about 100 mg of Oxaprozin to a 100-mL volumetric flask. Add 2 mL of methylene chloride, 2 mL of water, and 75 mL of acetonitrile. Sonicate after each solvent is added. Dilute with acetonitrile to volume. [NOTE—This is used to monitor all known and unknown impurities, except imidazolic acid and oximide.]

Sample solution B: 1 mg/mL of Oxaprozin in Diluent B. [NOTE—This is used to monitor only imidazolic acid and oximide.]

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 238 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: Standard solution and System suitability solution

[NOTE—The relative retention times for oxaprozin and benzil are about 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 3.0 between oxaprozin and benzil, System suitability solution

Tailing factor: NMT 2.0, Standard solution

Relative standard deviation: NMT 5.0%, Standard solution

Analysis

Samples: Sample solution A and Sample solution B
Calculate the percentage of imidazolic acid and oximide in the portion of Oxaprozin taken:

$$\text{Result} = (r_U/r_T) \times F \times 100$$

r_U = peak areas of imidazolic acid or oximide from Sample solution B

r_T = sum of the peak areas from Sample solution B

F = relative response factor (see Table 2)

Calculate the percentage of any other impurity in the portion of Oxaprozin taken:

$$\text{Result} = (r_U/r_T) \times F \times 100$$

r_U = peak area of each other impurity from Sample solution A

r_T = sum of the peak areas from Sample solution A

F = relative response factor (see Table 2)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Imidazolic acid	0.14	1.15	0.1
Unidentified impurity 1	0.42	1.21	0.1
Oximide	0.73	0.91	0.1
Unidentified impurity 2	0.84	0.85	0.1
Unidentified impurity 3	1.08	1.29	0.1
Unidentified impurity 4	1.50	1.46	0.1
Unidentified impurity 5	1.57	2.09	0.1
Total impurities	—	—	0.5

[NOTE—The values of F for all known impurities, except imidazolic acid and oximide, are found using Sample solution A. The values of F for imidazolic acid and oximide are found using Sample solution B.]

SPECIFIC TESTS• **LOSS ON DRYING (731)**

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Oxaprozin RS

Oxaprozin Tablets**DEFINITION**

Oxaprozin Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of oxaprozin ($C_{18}H_{15}NO_3$).

[NOTE—Because of light sensitivity, protect all oxaprozin samples and standard solutions from light.]

IDENTIFICATION• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

Sample solution: 2 mg/mL of oxaprozin in acetone

Developing solvent system: Ethyl acetate and glacial acetic acid (99:1)

Acceptance criteria: Meet the requirements

• **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

ASSAY• **PROCEDURE**

Solution A: 0.1% Phosphoric acid. Add phosphoric acid, dropwise, to water to obtain a pH of 2.00 ± 0.10 .

Mobile phase: Acetonitrile and Solution A (45:55)

Standard solution: Dissolve an accurately weighed quantity of USP Oxaprozin RS in acetonitrile to obtain a solution having a concentration of about 12 µg/mL of oxaprozin.

Sample stock solution: Nominally 0.6 mg/mL of oxaprozin prepared as follows. Transfer a suitable amount of oxaprozin from NLT 20 powdered Tablets to an appropriate volumetric flask. Add water to 10% of the final volume, and sonicate for 10 min. Add 40% of the final volume of acetonitrile, and sonicate for 30 min. Shake by mechanical means for an additional 30 min.

Add 30% of the final volume of acetonitrile, and sonicate for 10 min. Dilute with acetonitrile to volume. Pass through a suitable filter. Use the filtrate.

Sample solution: Nominally equivalent to 12 µg/mL of oxaprozin from the *Sample stock solution* in acetonitrile

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxaprozin (C₁₈H₁₅NO₃) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Oxaprozin RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of oxaprozin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.05 M monobasic potassium phosphate buffer, pH 7.4; 1000 mL

Apparatus 2: 75 rpm

Time: 45 min

Detector: UV 286 nm (maximum absorbance)

Standard solution: A known concentration of USP Oxaprozin RS in *Medium*. [NOTE—A quantity of methanol, not exceeding 5% of the final volume, can be added to help solubilize the USP Reference Standard.]

Sample solution: Filter portions of the solution under test, suitably diluted with *Medium*, if necessary.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxaprozin (C₁₈H₁₅NO₃) dissolved by using UV absorption from the *Sample solution* in comparison with the *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of oxaprozin (C₁₈H₁₅NO₃) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Solution A and Mobile phase: Proceed as directed in the Assay.

Standard solution: 0.001 mg/mL of USP Oxaprozin RS in acetonitrile

Sample solution: Nominally 1 mg/mL of oxaprozin prepared as follows. Transfer a suitable amount of oxaprozin from NLT 20 powdered Tablets to an appropriate volumetric flask. Add water to 10% of the final volume, and sonicate for 10 min. Add 40% of the final volume of acetonitrile, and sonicate for 30 min. Shake by mechanical means for an additional 30 min. Add 30% of the final volume of acetonitrile, and sonicate for 10 min. Dilute with acetonitrile to volume. Pass through a suitable filter. Use the filtrate.

Chromatographic system: Proceed as directed in the Assay except use a column temperature of 20°.

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentages of benzoic acid, benzil, and each individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of benzoic acid, benzil, or each individual unspecified degradation product from the *Sample solution*

r_s = peak response of oxaprozin from the *Standard solution*

C_s = concentration of USP Oxaprozin RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of oxaprozin in the *Sample solution* (mg/mL)

F = relative response factor (see Table 1)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzoic acid	0.6	0.12	0.2
Oxaprozin	1.0	—	—
Benzil	1.9	0.62	0.2
Any individual unspecified degradation product	—	1.0	0.2

*2-Hydroxy-1,2-diphenylethan-1-one.

SPECIFIC TESTS

• WATER DETERMINATION, Method 1a (921):

NMT 3.5%

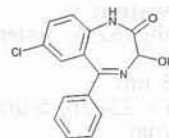
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Oxaprozin RS

Oxazepam



C₁₅H₁₁ClN₂O₂ 286.71

2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-, (±)-;

(±)-7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2H-1,4-benzodiazepin-2-one [604-75-1].

DEFINITION

Oxazepam contains NLT 98.0% and NMT 102.0% of oxazepam (C₁₅H₁₁ClN₂O₂), calculated on the dried basis.

IDENTIFICATION• **A. INFRARED ABSORPTION (197K)**• **B. ULTRAVIOLET ABSORPTION (197U)**

Sample solution: 4 µg/mL in alcohol

Analytical wavelength: 229 nm

Acceptance criteria: Absorptivities, calculated on the dried basis, differ by NMT 3.0%.

ASSAY• **PROCEDURE**

Sample solution: Transfer about 400 mg of Oxazepam, accurately weighed, to a beaker, and dissolve in 100 mL of dimethylformamide.

Analysis: Titrate the Sample solution with 0.1 N tetrabutylammonium hydroxide VS. Determine the endpoint potentiometrically, using a calomel-glass electrode system and taking precautions against absorption of atmospheric carbon dioxide. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 28.67 mg of oxazepam ($C_{15}H_{11}ClN_2O_2$).

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES• **RESIDUE ON IGNITION (281):** NMT 0.3%• **ORGANIC IMPURITIES**

Prepare the solutions immediately before use.

Diluent: Acetonitrile and water (50:50)

Solution A: Dissolve 3.5 g of dipotassium hydrogen phosphate in 900 mL of water. Adjust with 1 N sodium hydroxide to a pH of 10.5, and dilute with water to 1 L.

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
4	75	25
34	25	75
45	25	75
50	75	25
60	75	25

System suitability solution: 1.6 µg/mL of USP Oxazepam Related Compound A RS and USP Chlordiazepoxide Related Compound A RS and 0.8 mg/mL of USP Oxazepam RS in Diluent

Standard solution: 1.6 µg/mL of USP Oxazepam RS in Diluent

Sample solution: 0.8 mg/mL of Oxazepam in Diluent

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: System suitability solution and Standard solution

Suitability requirements

Resolution: NLT 1.5 between chlordiazepoxide related compound A and oxazepam related compound A, System suitability solution

Relative standard deviation: NMT 5.0%, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Oxazepam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak area of any impurity from the Sample solution r_S = peak area of oxazepam from the Standard solution C_S = concentration of USP Oxazepam RS in the Standard solution (mg/mL) C_U = concentration of oxazepam in the Sample solution (mg/mL) F = relative response factor, see Table 2

Acceptance criteria: See Table 2. Disregard any peak representing less than 0.05% of the area of the main peak.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chlordiazepoxide related compound A	0.7	1.0	0.2
Oxazepam related compound A	0.8	0.25	0.2
Oxazepam	1.0	—	—
Oxazepam related compound B ^a	1.2	0.90	0.2
Oxazepam related compound C ^b	1.4	1.0	0.2
Oxazepam related compound D ^c	2.0	1.0	0.2
Any individual unknown impurity	—	—	0.10
Total impurities	—	—	1.0

^a 7-Chloro-2-oxo-5-phenyl-2,3-dihydro-1H-benzodiazepin-3-yl acetate.^b 6-Chloro-4-phenylquinazoline-2-carbaldehyde.^c 5-Chloro-2-aminobenzophenone.**SPECIFIC TESTS**• **pH (791)**

Sample: A suspension of 1 g of Oxazepam in 50 mL water

Acceptance criteria: 4.8–7.0

• **LOSS ON DRYING (731)**

Analysis: Dry at a pressure below 5 mm of mercury at 105° for 3 h.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in well-closed containers.• **USP REFERENCE STANDARDS (11)**

USP Chlordiazepoxide Related Compound A RS

7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.

 $C_{15}H_{11}ClN_2O_2$ 286.71

USP Oxazepam RS

USP Oxazepam Related Compound A RS

7-Chloro-5-phenyl-4,5-dihydro-1H-benzodiazepine-2,3-dione.

 $C_{15}H_{11}ClN_2O_2$ 286.71**Oxazepam Capsules****DEFINITION**Oxazepam Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{15}H_{11}ClN_2O_2$.

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY• **PROCEDURE**

Diluent: Methanol and water (9:1)

Buffer: 8.5 g/L of monobasic potassium phosphate in water. Adjust with 1 N sodium hydroxide to a pH of 6.5.

Mobile phase: Methanol and Buffer (3:2)

Standard solution: 0.1 mg/mL of USP Oxazepam RS in Diluent

Sample solution: 0.1 mg/mL of oxazepam in Diluent, from the contents of NLT 20 Capsules. [NOTE—Sonicate for 15 min and shake for 15 min. Pass through a filter of 0.45- μ m pore size.]

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 232 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection size: 20 μ L

Run time: At least 1.7 times the retention time of oxazepam

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of $C_{15}H_{11}ClN_2O_2$ in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxazepam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxazepam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: 0.1 N hydrochloric acid; 1000 mL

Apparatus 2: 75 rpm

Time: 60 min

Standard stock solution: 0.1 mg/mL of USP Oxazepam RS in methanol

[NOTE—Prepare NMT 30 min before use.]

Standard solution: 0.01 mg/mL of USP Oxazepam RS in Medium from the Standard stock solution. [NOTE—Keep it at about 6° for the Analysis. This solution is stable for 72 h if kept refrigerated.]

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size. Keep it at about 6° for the Analysis.

Mobile phase: Methanol, water, and glacial acetic acid (60:40:1)

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 232 nm

Column: 4-mm \times 15-cm; packing L7

Flow rate: 2 mL/min

Injection size: 20 μ L

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 1.5 for the oxazepam peak

Relative standard deviation: NMT 3.0%

Analysis

Samples: Standard solution and Sample solution

Tolerances: NLT 75% (Q) of the labeled amount of $C_{15}H_{11}ClN_2O_2$ is dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES**Organic Impurities**• **PROCEDURE**

Diluent, Buffer, and Mobile phase: Proceed as directed in the Assay.

Standard solution: 2 μ g/mL of USP Oxazepam RS in Diluent

Sample solution: 0.2 mg/mL of oxazepam in Diluent, from the contents of NLT 20 Capsules. [NOTE—Sonicate for 15 min and shake for 15 min. Pass through a filter of 0.45- μ m pore size.]

Chromatographic system: Proceed as directed in the Assay.

Run time: 3.5 times the retention time of oxazepam

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of oxazepam from the *Standard solution*

C_S = concentration of USP Oxazepam RS in the *Standard solution* (μ g/mL)

C_U = nominal concentration of oxazepam in the *Sample solution* (μ g/mL)

Acceptance criteria

Individual impurities: See Impurity Table 1. [NOTE—Disregard peaks less than 0.05%.]

Total impurities: NMT 0.5%, not including 2-amino 5-chlorobenzophenone

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxazepam	1.0	—
2-Amino 5-chlorobenzophenone	2.7	0.5
Any individual unspecified degradation product	—	0.1

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**
USP Oxazepam RS

Oxazepam Tablets

DEFINITION

Oxazepam Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxazepam ($C_{15}H_{11}ClN_2O_2$).

IDENTIFICATION

- **A.** The *Sample solution* in the Assay exhibits a maximum absorbance at 229 ± 2 nm.

ASSAY

• PROCEDURE

Standard solution: 4 µg/mL of USP Oxazepam RS in alcohol

Sample stock solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to 50 mg of oxazepam, to a medium-pore size, sintered-glass funnel that is fitted into a small suction flask. Add 25 mL of alcohol, mix with the aid of a stirring rod, and after about 5 min apply gentle suction to remove the extract. Repeat the extraction with four additional 25-mL portions of alcohol, transfer the extracts to a 250-mL volumetric flask, and dilute with alcohol to volume.

Sample solution: Dilute 2.0 mL of *Sample stock solution* with alcohol to 100 mL.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 229 nm

Cell: 1 cm

Blank: Alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxazepam ($C_{15}H_{11}ClN_2O_2$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Oxazepam RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of oxazepam in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 1000 mL

Apparatus 2: 50 rpm

Time: 60 min

Mobile phase: Methanol, glacial acetic acid, and water (60:1:40)

Sample solution: Pass a portion of the solution under test through a suitable filter.

Standard solution: Prepare a known concentration of USP Oxazepam RS in *Medium* at a known concentration similar to the *Sample solution*. [NOTE—A volume of alcohol not to exceed 10% of the final total volume may be used to dissolve USP Oxazepam RS.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 232 nm

Column: 4-mm × 30-cm; packing L7

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxazepam ($C_{15}H_{11}ClN_2O_2$) dissolved, using the peak response of oxazepam from the *Sample solution* in comparison to that from the *Standard solution*.

Tolerances: NLT 80% (Q) of the labeled amount of oxazepam ($C_{15}H_{11}ClN_2O_2$) is dissolved.

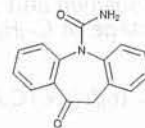
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**
USP Oxazepam RS

Oxcarbazepine



$C_{15}H_{12}N_2O_2$ 252.27
5*H*-Dibenz[*b,f*]azepine-5-carboxamide, 10,11-dihydro-10-oxo-;
10,11-Dihydro-10-oxo-5*H*-dibenz[*b,f*]azepine-5-carboxamide
[28721-07-5].

DEFINITION

Oxcarbazepine contains NLT 98.0% and NMT 102.0% of oxcarbazepine ($C_{15}H_{12}N_2O_2$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

[NOTE—If the spectrum obtained shows differences, dissolve the substance to be examined in chloroform, and evaporate to dryness. Compare the spectrum of the residue to that of a similarly prepared USP Oxcarbazepine RS.]

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Buffer: 6.8 g/L of monobasic potassium phosphate in water. For each liter prepared, add 2 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 6.0 ± 0.1 .

Mobile phase: Methanol, acetonitrile, and *Buffer* (11:8:31)

Standard solution: 0.1 mg/mL of USP Oxcarbazepine RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Oxcarbazepine in *Mobile phase*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 215 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Column temperature:** 50°**Flow rate:** 1.5 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of oxcarbazepine (C₁₅H₁₂N₂O₂) in the portion of Oxcarbazepine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL) C_U = concentration of Oxcarbazepine in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 10 ppm (Official 1-Jan-2018)

- **ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—If oxcarbazepine related compound A and oxcarbazepine related compound B are known process impurities, *Organic Impurities, Procedure 2* is recommended.]**Mobile phase:** Prepare as directed in the *Assay*.**System suitability solution:** 0.1 mg/mL each of USP Oxcarbazepine RS and USP Carbamazepine RS in *Mobile phase***Standard solution:** 0.25 μg/mL of USP Oxcarbazepine RS in *Mobile phase***Sample solution:** 0.5 mg/mL of Oxcarbazepine in *Mobile phase***Chromatographic system:** Proceed as directed in the *Assay*, except to use a run time 10 times the retention time of oxcarbazepine.**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 8.0 between oxcarbazepine and carbamazepine, *System suitability solution***Relative standard deviation:** NMT 10.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oxcarbazepine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_S = peak response of oxcarbazepine from the *Standard solution* C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL) C_U = concentration of Oxcarbazepine in the *Sample solution* (mg/mL) F = relative response factor (see *Table 1*)**Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxcarbazepine	1.0	1.0	—
Carbamazepine ^a	1.7	1.9	0.5
Oxcarbazepine related compound E	2.1	1.2	0.05
Methoxycarbamazepine ^b	2.5	1.6	0.05
Carbamazepine related compound B ^c	7.4	1.3	0.05
Methoxydibenzazepine ^d	7.9	1.5	0.05
Any individual unspecified impurity	—	1.0	0.05
Total impurities	—	—	1.0

^a 5*H*-Dibenz[*b*,*f*]azepine-5-carboxamide.^b 10-Methoxy-5*H*-Dibenz[*b*,*f*]azepine-5-carboxamide.^c 5*H*-Dibenz[*b*,*f*]azepine.^d 10-Methoxy-5*H*-Dibenz[*b*,*f*]azepine.• **ORGANIC IMPURITIES, PROCEDURE 2****Buffer A:** 0.004 mol/L of monobasic potassium phosphate and 0.063 mol/L of dibasic sodium phosphate**Buffer B:** To 1 L of 3.6 g/L edetate disodium in water add 1 L of *Buffer A*.**Diluent:** 1.8 g/L of ascorbic acid in water**Solution A:** Acetonitrile, tetrahydrofuran, *Buffer B*, and water (1:2:2:15)**Solution B:** Acetonitrile, tetrahydrofuran, *Buffer B*, and water (6:1:1:2)**Mobile phase:** See *Table 2*.**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	80	20
1	80	20
29	30	70
30	30	70
33	80	20
42	80	20

System suitability solution: 2 μg/mL each of USP Oxcarbazepine Related Compound A RS, USP Oxcarbazepine Related Compound B RS, USP Oxcarbazepine Related Compound D RS, and USP Oxcarbazepine Related Compound E RS in a 1:1 mixture of acetonitrile and *Diluent***Standard stock solution:** 0.1 mg/mL of USP Oxcarbazepine RS in acetonitrile**Standard solution:** 2 μg/mL of USP Oxcarbazepine RS in a 1:1 mixture of acetonitrile and *Diluent***Sample solution:** 1.0 mg/mL of Oxcarbazepine in a 1:1 mixture of acetonitrile and *Diluent***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 240 nm**Column:** 4.6-mm × 25-cm; 3-μm packing L1**Column temperature:** 50°**Flow rate:** 0.8 mL/min**Injection volume:** 50 μL**System suitability****Samples:** *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between oxcarbazepine related compound A and oxcarbazepine related compound B; NLT 1.2 between oxcarbazepine related compound D and oxcarbazepine related compound E, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oxcarbazepine taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each impurity from the *Sample solution*

r_s = peak response of oxcarbazepine from the *Standard solution*

C_s = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)

C_u = concentration of Oxcarbazepine in the *Sample solution* (mg/mL)

F = relative response factor (see Table 3)

Acceptance criteria: See Table 3. [NOTE—Disregard any peak below 0.03%.]

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxcarbazepine related compound F ^a	0.76	0.59	0.2
Oxcarbazepine	1.0	—	—
N-Carbamoyl oxcarbazepine ^b	1.1	0.91	0.05
Oxcarbazepine related compound A ^c	1.2	1.1	0.2
Oxcarbazepine related compound B ^d	1.3	1.1	0.1
Dibenzazepinodione ^e	1.7	2.0	0.1
Oxcarbazepine related compound D ^f	2.3	1.7	0.2
Oxcarbazepine related compound E	2.4	3.3	0.05
Any individual unspecified impurity	—	1.0	0.05
Total impurities	—	—	1.0

^a 10,11-Dioxo-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide.

^b N-Carbamoyl-10-oxo-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide.

^c N-Formyl-10-oxo-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide.

^d N-Acetyl-10-oxo-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide.

^e 5H-Dibenzo[b,f]azepine-10,11-dione.

^f 10-(10-Oxo-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamido)-5H-dibenzo[b,f]azepine-5-carboxamide.

SPECIFIC TESTS

- **WATER DETERMINATION, Method 1a (921):** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.

• **USP REFERENCE STANDARDS (11)**

USP Oxcarbazepine RS

USP Carbamazepine RS

USP Oxcarbazepine Related Compound A RS

N-Formyl-10-oxo-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide.

C₁₆H₁₂N₂O₃ 280.28

USP Oxcarbazepine Related Compound B RS

N-Acetyl-10-oxo-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide.

C₁₇H₁₄N₂O₃ 294.30

USP Oxcarbazepine Related Compound D RS

10-(10-Oxo-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamido)-5H-dibenzo[b,f]azepine-5-carboxamide.

C₃₀H₂₂N₄O₃ 486.52

USP Oxcarbazepine Related Compound E RS

10(11H)-Oxo-5H-Dibenz[b,f]azepine.

C₁₄H₁₁NO 209.24

Oxcarbazepine Oral Suspension**DEFINITION**

Oxcarbazepine Oral Suspension contains NLT 95.0% and NMT 105.0% of the labeled amount of oxcarbazepine (C₁₅H₁₂N₂O₂).

IDENTIFICATION

- **A.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Protect all solutions from light.

Buffer: Dissolve 1.36 g of sodium acetate trihydrate and 0.6 g of glacial acetic acid in 1 L of water. Adjust with glacial acetic acid to a pH of 4.4.

Solution A: Acetonitrile, tetrahydrofuran, *tert*-butyl methyl ether, and *Buffer* (130:30:9:830)

Solution B: Acetonitrile, tetrahydrofuran, *tert*-butyl methyl ether, and *Buffer* (670:30:9:290)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93	7
2	90	10
10	90	10
25	10	90
26	93	7
35	93	7

Diluent: Dissolve 0.1 g of ascorbic acid and 1 mL of acetonitrile in 1 L of water.

Standard stock solution: 1 mg/mL of USP Oxcarbazepine RS in acetonitrile. Sonicate to aid in dissolution.

Standard solution: 0.25 mg/mL of USP Oxcarbazepine RS from the *Standard stock solution*, prepared as follows. Dilute a suitable volume of the *Standard stock solution* first in *Diluent*, using 70% final volume. Allow the solution to equilibrate to room temperature, and dilute with acetonitrile to volume.

Sample solution: 0.25 mg/mL of oxcarbazepine from a portion of Oral Suspension, prepared as follows. Dissolve first in *Diluent* using 8% of final volume, fill 30% of final volume with acetonitrile. Sonicate for 15 min. Add *Diluent* to fill 36% of final volume. Shake the flask

vigorously. Allow the solution to equilibrate to room temperature, and dilute with *Diluent* to volume.

System suitability stock solution: 0.01 mg/mL of USP Oxcarbazepine Related Compound A RS and 0.02 mg/mL of USP Oxcarbazepine Related Compound C RS in acetonitrile

System suitability solution: 0.5 µg/mL of USP Oxcarbazepine Related Compound A RS and 1 µg/mL of USP Oxcarbazepine Related Compound C RS from the *System suitability stock solution*, in *Standard solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.0-mm × 25-cm; 3-µm packing L1

Column temperature: 50°

Flow rate: 0.6 mL/min

Injection volume: 5 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—Refer to *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.3 between oxcarbazepine related compound C and oxcarbazepine related compound A, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxcarbazepine (C₁₅H₁₂N₂O₂) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxcarbazepine from the *Sample solution*

r_S = peak response of oxcarbazepine from the *Standard solution*

C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 1% sodium dodecyl sulfate in water; 890 mL

Apparatus 2: 75 rpm

Time: 30 min

Analysis: Shake manually a bottle of Oral Suspension for about 20 s. Using a 10-mL syringe, draw 10.0 mL of the Oral Suspension. Attach a long needle to the syringe. Deliver carefully 10.0 mL of Oral Suspension through the needle to the bottom of the vessel containing preheated *Medium*. Take about 10 mL of the *Medium* from the vessel to clean the syringe, and transfer it back to the vessel. Start the paddle rotation immediately after introduction of each sample.

Mobile phase: Methanol, acetic acid, and water (24:1:75)

Standard solution: 0.7 mg/mL of USP Oxcarbazepine RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter of 1-µm pore size, discard the first few mL.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 310 nm

Column: 4.6-mm × 25-cm; 10-µm packing L10

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxcarbazepine (C₁₅H₁₂N₂O₂) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)

L = label claim (mg in 10 mL)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of oxcarbazepine (C₁₅H₁₂N₂O₂) is dissolved.

• **DELIVERABLE VOLUME** (698): Meets the requirements

IMPURITIES

• ORGANIC IMPURITIES

Protect all solutions from light.

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard stock solution: 0.5 mg/mL of USP Carbamazepine RS in acetonitrile. Sonicate to aid in dissolution.

Standard solution: 0.5 µg/mL of USP Carbamazepine RS from the *Standard stock solution* prepared as follows. Dilute a volume of the *Standard stock solution* first in *Diluent*, using 70% of final volume. Cool to room temperature, and dilute with acetonitrile to volume.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.3 between oxcarbazepine related compound C and oxcarbazepine related compound A peaks, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of carbamazepine from the *Standard solution*

C_S = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Acridine carboxylic acid ^a	0.24	11.1	0.1
Carbamazepinedione ^b	0.65	0.68	0.2
Oxcarbazepine	1.0	1.0	—
Oxcarbazepine related compound C ^c	1.33	12.5	0.1
Oxcarbazepine related compound A ^{d,e}	1.38	—	—
Carbamazepine	1.66	1.0	—
Dibenzazepinodione ^f	1.97	1.1	0.2
Acridine ^g	2.49	11.1	0.1
Dibenzazepinone ^h	2.62	2.9	0.1
Any unspecified individual degradation product	—	1.0	0.1
Total impurities	—	—	0.8

^a Acridine-9-carboxylic acid.^b 10,11-Dioxo-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine-5-carboxamide.^c Acridin-9(10*H*)-one.^d *N*-Formyl-10-oxo-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine-5-carboxamide.^e For system suitability purposes only.^f 5*H*-Dibenzo[*b,f*]azepine-10,11-dione.^g Acridine.^h 10(11*H*)-Oxo-5*H*-dibenz[*b,f*]azepine.**SPECIFIC TESTS**• **pH (791):** 2.5–3.7• **MICROBIAL ENUMERATION TESTS (61) and TEST FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed 10² cfu/mL. The total yeasts and molds count does not exceed 10¹ cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in light-resistant containers. Store at controlled room temperature.• **USP REFERENCE STANDARDS (11)**

USP Carbamazepine RS

USP Oxcarbazepine RS

USP Oxcarbazepine Related Compound A RS

N-Formyl-10-oxo-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine-5-carboxamide.C₁₆H₁₂N₂O₃ 280.28

USP Oxcarbazepine Related Compound C RS

Acridin-9(10*H*)-one.C₁₃H₉NO 195.22**Oxcarbazepine Tablets****DEFINITION**Oxcarbazepine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxcarbazepine (C₁₅H₁₂N₂O₂).**IDENTIFICATION**• **A. INFRARED ABSORPTION (197K)**

Sample: Weigh about 840 mg of crushed Tablet powder, and add to a 50-mL volumetric flask. Add 45 mL of chloroform, and shake the flask for 30 min on a mechanical shaker. Add chloroform to volume, centrifuge, and collect the supernatant in a Petri dish. Evaporate the supernatant on a water bath at 60°. Dry the residue, and crush the residue thoroughly with potassium bromide in the ratio of 1:100.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Add 2 mL of triethylamine. Adjust with phosphoric acid to a pH of 6.0.

Diluent: Methanol and water (80:20)

Mobile phase: Methanol, acetonitrile, and *Buffer* (22:16:62)

Standard stock solution: 0.5 mg/mL of USP Oxcarbazepine RS in *Diluent*. Sonication may be used to aid in dissolution.

Standard solution: 0.1 mg/mL of USP Oxcarbazepine RS in *Mobile phase* from the *Standard stock solution*

Sample stock solution: Nominally equivalent to 1.2 mg/mL of oxcarbazepine from a portion of finely powdered (NLT 20) Tablets, prepared as follows. Transfer a weighed quantity of powdered Tablets, equivalent to 600 mg of oxcarbazepine, to a 500-mL volumetric flask. Add *Diluent* to fill 50% of the final volume. Sonicate for 15 min with intermittent swirling, cool to room temperature, and dilute with *Diluent* to volume. Pass this solution through a suitable 2-μm glass filter, and discard the first portion of the filtrate.

Sample solution: 0.1 mg/mL of oxcarbazepine in *Mobile phase* from a portion of the filtrate obtained from the *Sample stock solution*

Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μL

Temperatures

Sample: 5°

Column: 50°

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxcarbazepine (C₁₅H₁₂N₂O₂) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Test 1

Medium

For Tablets labeled to contain 150 mg: 0.3% (w/v)

sodium dodecyl sulfate in water; 900 mL, deaerated

For Tablets labeled to contain 300 mg: 0.6% (w/v)

sodium dodecyl sulfate in water; 900 mL, deaerated

For Tablets labeled to contain 600 mg: 1.0% (w/v)

sodium dodecyl sulfate in water; 900 mL, deaerated

Apparatus 2: 60 rpm

Times: 30 and 60 min

Standard stock solution: 0.35 mg/mL of USP Oxcarbazepine RS in methanol

Standard solution: Dilute the *Standard stock solution* with the corresponding *Medium* to obtain a final concentration of 0.0175 mg/mL of USP Oxcarbazepine RS.

Sample solution: Use portions of the solution under test passed through a suitable filter of 0.45- μ m pore size. The volume of the solution under test withdrawn must be replaced by the same volume of corresponding *Medium*. Dilute with the appropriate *Medium* if necessary, according to the Tablet strength, to obtain a final concentration similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 256 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$) dissolved at 30 min (Q_{30}):

$$Q_{30} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

Calculate the percentage of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$) dissolved at 60 min (Q_{60}):

$$Q_{60} = [(A_U/A_S) \times (C_S/L) \times D \times V \times 100] + [Q_{30} \times (V_S/V)]$$

A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)
 D = dilution factor of the *Sample solution*
 V = volume of *Medium*, 900 mL
 V_S = volume of the solution under test withdrawn (mL)

Tolerances: NLT 70% (Q) of the labeled amount of oxcarbazepine is dissolved in 30 min, and NLT 80% (Q) of the labeled amount of oxcarbazepine is dissolved in 60 min.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium

For Tablets labeled to contain 150 mg: 0.3% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated

For Tablets labeled to contain 300 mg: 0.6% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated

For Tablets labeled to contain 600 mg: 1.0% (w/v) sodium dodecyl sulfate in water; 900 mL, deaerated

Apparatus 2: 60 rpm

Times: 30 and 60 min

Standard stock solution: 3.3 mg/mL of USP Oxcarbazepine RS in methanol. [NOTE—This solution is stable for 22 h at 10°.]

Standard solution: Dilute the *Standard stock solution* with the corresponding *Medium*, according to the Tablet strength, to obtain a final concentration of ($L/900$) mg/mL, where L is the label claim in mg/Tablet.

Sample solution: Use portions of the solution under test passed through a suitable filter of 0.45- μ m pore size. The volume of the solution under test withdrawn must be replaced by the same volume of corresponding *Medium*.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 304 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$) dissolved at 30 min (Q_{30}):

$$Q_{30} = (A_U/A_S) \times (C_S/L) \times D \times V \times 100$$

Calculate the percentage of the labeled amount of oxcarbazepine ($C_{15}H_{12}N_2O_2$) dissolved at 60 min (Q_{60}):

$$Q_{60} = [(A_U/A_S) \times (C_S/L) \times D \times V \times 100] + [Q_{30} \times (V_S/V)]$$

A_U = absorbance of the *Sample solution*
 A_S = absorbance of the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)
 D = dilution factor of the *Sample solution*
 V = volume of *Medium*, 900 mL
 V_S = volume of the solution under test withdrawn (mL)

Tolerances

For Tablets labeled to contain 150 or 300 mg: NLT 70% (Q) of the labeled amount of oxcarbazepine is dissolved in 30 min, and NLT 80% (Q) of the labeled amount of oxcarbazepine is dissolved in 60 min.

For Tablets labeled to contain 600 mg: NLT 50% (Q) of the labeled amount of oxcarbazepine is dissolved in 30 min, and NLT 80% (Q) of the labeled amount of oxcarbazepine is dissolved in 60 min.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

[NOTE—On the basis of the synthetic route, perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*. If methoxycarbamazepine is a potential degradation product, *Procedure 1* is recommended. If carbamazepinedione or dibenzazepinodione is a potential degradation product, *Procedure 2* is recommended.]

• ORGANIC IMPURITIES, PROCEDURE 1

Buffer and Chromatographic system: Proceed as directed in the *Assay*.

Diluent: Methanol and water (60:40)

Mobile phase: Methanol, acetonitrile, and *Buffer* (29:21:75)

System suitability solution: 0.5 mg/mL of USP Oxcarbazepine RS and 1.0 μ g/mL of USP Carbamazepine RS in *Mobile phase*

Standard solution: 0.5 μ g/mL of USP Oxcarbazepine RS in *Mobile phase*

Sample stock solution: Nominally equivalent to 1.2 mg/mL of oxcarbazepine from a portion of finely powdered (NLT 20) Tablets, prepared as follows. Transfer a weighed quantity of powdered Tablets, equivalent to 600 mg of oxcarbazepine, to a 500-mL volumetric flask. Add *Diluent* to fill 50% of the final volume. Sonicate for 15 min with intermittent swirling, cool to room temperature, and dilute with *Diluent* to volume. Pass this solution through a suitable 2- μ m glass filter, and discard the first portion of the filtrate.

Sample solution: 0.5 mg/mL of oxcarbazepine from the *Sample stock solution* in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 8.0 between oxcarbazepine and carbamazepine, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of oxcarbazepine from the *Standard solution*

- C_s = concentration of USP Oxcarbazepine RS in the *Standard solution* (mg/mL)
 C_u = nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)
 F = relative response factor for the corresponding impurity (see Table 1)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxcarbazepine	1.0	1.0	—
Carbamazepine	1.6	1.5	0.5
Dibenzazepinone ^a	2.0	1.0	0.05
Methoxy-carbamazepine ^b	2.3	1.3	0.05
Any unspecified individual degradation product	—	1.0	0.10
Total impurities	—	—	0.75

^a 10-(11H)-Oxo-5H-dibenz[b,f]azepine.

^b 10-Methoxy-5H-dibenz[b,f]azepine-5-carboxamide.

• ORGANIC IMPURITIES, PROCEDURE 2

Buffer A: 4.2 g of tris(hydroxymethyl)amino methane and 0.2 g of edetate disodium in 1 L of water

Buffer B: 18 g of tris(hydroxymethyl)amino methane and 0.9 g of edetate disodium in 1 L of water

Diluent: Acetonitrile and 1.8 g/L of ascorbic acid in water (1:99)

Solution A: Acetonitrile, tetrahydrofuran, and Buffer A (5:10:85)

Solution B: Acetonitrile, tetrahydrofuran, and Buffer B (70:10:20)

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	95	5
33.0	30	70
33.1	95	5
45.0	95	5

System suitability stock solution: 1 µg/mL of USP Oxcarbazepine Related Compound C RS and 12 µg/mL of USP Carbamazepine RS in acetonitrile. Sonication may be used to aid in dissolution. [NOTE—The water bath temperature should not exceed 23°.]

System suitability solution: 0.05 µg/mL of USP Oxcarbazepine Related Compound C RS, 0.6 µg/mL of USP Carbamazepine RS, and 0.06 mg/mL of USP Oxcarbazepine RS prepared as follows. Transfer a suitable volume of *System suitability stock solution* to a volumetric flask containing 50% of the final volume of *Diluent*. Allow the solution to reach ambient temperature, and dilute with acetonitrile to volume.

Standard stock solution: 12 µg/mL of USP Carbamazepine RS in acetonitrile. Sonication may be used to aid in dissolution. [NOTE—The water bath temperature should not exceed 23°.]

Standard solution: 0.6 µg/mL of USP Carbamazepine RS from *Standard stock solution* prepared as follows. Transfer a suitable volume of *Standard stock solution* to a flask containing 50% of the final volume of *Diluent* and 20% of the final volume of acetonitrile. Allow the solution to reach ambient temperature, and dilute with acetonitrile to volume.

Sample stock solution: 1.5 mg/mL of oxcarbazepine from a portion of finely powdered (NLT 20) Tablets prepared as follows. Transfer a weighed quantity of powdered Tablets, equivalent to about 375 mg of oxcarbazepine, to a 250-mL volumetric flask. Add 150 mL of acetonitrile, and sonicate for 15 min. Shake for 15 min, and dilute with acetonitrile to volume. Mix, and allow the suspension to settle for 30 min. Use the supernatant. [NOTE—The water bath temperature should not exceed 23°.]

Sample solution: 0.3 mg/mL of oxcarbazepine from the *Sample stock solution* prepared as follows. Transfer a suitable volume of *Sample stock solution* to a volumetric flask containing 50% of the final volume of *Diluent* and 20% of the final volume of acetonitrile. Allow the solution to warm to ambient temperature, and dilute with acetonitrile to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.0-mm × 25-cm; 5-µm packing L1

Flow rate: 0.5 mL/min

Injection volume: 20 µL

Temperatures

Sample: 5°

Column: 35°

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.2 between oxcarbazepine related compound C and carbamazepine, *System suitability solution*

Relative standard deviation: NMT 15%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each individual impurity from the *Sample solution*

r_s = peak response of carbamazepine from the *Standard solution*

C_s = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of oxcarbazepine in the *Sample solution* (mg/mL)

F = relative response factor for the corresponding impurity (see Table 3)

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Carbamazepinedione ^a	0.72	0.70	0.2
Oxcarbazepine	1.0	1.0	—
Oxcarbazepine related compound C ^b	1.3	—	—
Carbamazepine	1.4	1.0	0.5
Dibenzazepinodione ^c	1.7	2.8	0.2

^a 10,11-Dioxo-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide.

^b For system suitability and identification purposes only. Process impurity. Not included in total.

^c 5H-Dibenzo[b,f]azepine-10,11-dione.

Table 3 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any unspecified individual degradation product	—	1.0	0.1
Total impurities	—	—	1.0

^a 10,11-Dioxo-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide.

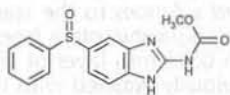
^b For system suitability and identification purposes only. Process impurity. Not included in total.

^c 5H-Dibenzo[b,f]azepine-10,11-dione.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.
- **USP REFERENCE STANDARDS (11)**
 - USP Carbamazepine RS
 - USP Oxcarbazepine RS
 - USP Oxcarbazepine Related Compound C RS
 - Acridin-9(10H)-one.
 - C₁₃H₉NO 195.22

Oxfendazole



C₁₅H₁₃N₃O₃S 315.35

Carbamic acid, 5-(phenylsulfinyl)-1H-benzimidazol-2-yl-, methyl ester.

Methyl 5-(phenylsulfinyl)-2-benzimidazolecarbamate [53716-50-0].

» Oxfendazole contains not less than 98.0 percent and not more than 100.5 percent of C₁₅H₁₃N₃O₃S, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Fenbendazole RS

USP Oxfendazole RS

Identification—

A: *Infrared Absorption* (197K).

B: The appearance of the principal spot in the chromatogram of the *Test solution* corresponds to that in the chromatogram of *Standard solution 1*, as obtained in the test for *Related compounds*.

Loss on drying (731)—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 105° for 2 hours: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.1%.

Related compounds—

Diluent—Prepare a mixture of ethyl acetate and glacial acetic acid (4:1).

Standard solution 1—Dissolve a quantity of USP Oxfendazole RS in *Diluent* to obtain a solution having a concentration of 0.1 mg per mL.

Standard solution 2—Dissolve a quantity of USP Fenbendazole RS in *Diluent* to obtain a solution having a concentration of 0.05 mg per mL.

Standard solution 3—Prepare a mixture of *Standard solution 1* and *Standard solution 2* (1:2).

Test solution—Dissolve 25 mg of Oxfendazole in *Diluent*, dilute with *Diluent* to 5 mL, and mix.

Procedure—Separately apply 20 µL portions of *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and the *Test solution* to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatograms in a solvent system consisting of a mixture of ethyl acetate and glacial acetic acid (95:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, and allow to air-dry. Examine the plate under short-wavelength UV light: the chromatogram obtained from *Standard solution 3* shows two clearly separated principal spots. In the chromatogram obtained from the *Test solution*, no spot corresponding to fenbendazole is more intense than the spot in the chromatogram obtained from *Standard solution 2* (1%), and no spot other than the principal spot and no spot corresponding to fenbendazole is more intense than the spot in the chromatogram obtained from *Standard solution 1* (2%).

Assay—Dissolve about 300 mg of Oxfendazole, accurately weighed, in 3 mL of anhydrous formic acid. Add 40 mL of acetic anhydride, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 31.54 mg of C₁₅H₁₃N₃O₃S.

Oxfendazole Oral Suspension

» Oxfendazole Oral Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of oxfendazole (C₁₅H₁₃N₃O₃S).

Packaging and storage—Preserve in tight containers, and protect from excessive heat.

Labeling—Label the Suspension to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Oxfendazole RS

Identification—The relative retention time of the oxfendazole peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

pH (791): between 4.3 and 4.9.

Assay—

Mobile phase—Prepare a solution of sodium acetate in water containing 2.5 mg per mL, and adjust with glacial acetic acid to a pH of 4.75 ± 0.1. Prepare a mixture of this solution and acetonitrile (800:225). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability solution—Prepare a solution in *Mobile phase* containing in each mL about 1.2 µg of methylparaben, 12 µg of sulfabenzamide, and 72 µg of USP Oxfendazole RS.

Internal standard solution—Prepare a solution of sulfabenzamide in *Mobile phase* containing about 0.3 mg per mL.

Standard preparation—Prepare a solution of USP Oxendazole RS in methanol having a known concentration of about 900 µg per mL. Transfer 20.0 mL of this solution to a 100-mL volumetric flask, add 4.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix. This solution contains about 180 µg of USP Oxendazole RS per mL.

Assay preparation—Transfer an accurately measured volume of the Suspension, previously well-mixed and free from air bubbles, equivalent to about 450 mg of oxendazole, to a 500-mL volumetric flask. Add about 30 mL of water, and swirl to disperse. Add about 300 mL of methanol, and dissolve with the aid of sonication. Transfer 20.0 mL of this solution to a 100-mL volumetric flask, add 4.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

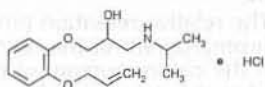
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector, a guard column containing packing L2, and a 4.6-mm × 25-cm analytical column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for sulfabenzamide, 0.8 for methylparaben, and 1.0 for oxendazole; the resolution, R_s , between the methylparaben peak and the oxendazole peak is not less than 2.0; the column efficiency determined from the oxendazole peak is not less than 2000 theoretical plates; and the relative standard deviation of replicate injections is not more than 1.5%. [NOTE—The detector sensitivity may be changed between the peaks to keep the responses on scale.]

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and measure the responses for the major peaks. Calculate the quantity, in mg, of oxendazole ($C_{15}H_{13}N_3O_3S$) in each mL of the Suspension taken by the formula:

$$2.5(C/V)(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Oxendazole RS in the *Standard preparation*; V is the volume, in mL, of Suspension taken to prepare the *Assay preparation*; and R_U and R_S are the ratios of the oxendazole peak response to the sulfabenzamide peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Oxprenolol Hydrochloride



$C_{15}H_{23}NO_3 \cdot HCl$ 301.81

2-Propanol, 1-(*o*-allyloxyphenoxy)-3-isopropylamino-, hydrochloride.

1-(*o*-Allyloxyphenoxy)-3-isopropylamino-2-propanol hydrochloride [6452-73-9].

» Oxprenolol Hydrochloride contains not less than 98.5 percent and not more than 101.0 percent of $C_{15}H_{23}NO_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Oxprenolol Hydrochloride RS

Clarity of solution—Dissolve 1 g in 10 mL of water: solution is clear.

Identification—

A: Infrared Absorption (197K).

B: A solution of it responds to the tests for *Chloride* (191).

pH (791): between 4.0 and 6.0, in a solution (1 in 10).

Loss on drying (731)—Dry about 3 g of it in vacuum at 60° for 6 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals, Method II** (231): 0.001%. • (Official 1-Jan-2018)

Chromatographic purity—

Diluting solvent—Prepare a mixture of chloroform and dehydrated alcohol (1:1).

Standard solution A—Prepare a solution of USP Oxprenolol Hydrochloride RS in *Diluting solvent* containing 20 mg per mL.

Standard solution B—Dilute an accurately measured volume of *Standard solution A* quantitatively, and stepwise if necessary, with *Diluting solvent* to obtain a solution containing 0.08 mg per mL.

Test solution—Transfer 200 mg of it to a 10-mL volumetric flask, dissolve in *Diluting solvent*, dilute with *Diluting solvent* to volume, and mix.

Procedure—Apply separate 5-µL portions of the *Test solution* and the *Standard solutions* to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, previously washed with methanol until the solvent front reaches the top of the plate, dried first in air and then at 100° for 20 minutes, and cooled in a desiccator. Allow the spots to dry. Line a suitable chromatographic chamber with filter paper, saturate the paper with 100 mL of a solvent system consisting of a mixture of ethyl acetate, glacial acetic acid, and water (15:5:5), and allow to stand for about 30 minutes. Place the plate in the chamber, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber and dry at 100° for 15 minutes. Spray the plate uniformly with a detection reagent consisting of a freshly prepared mixture of equal volumes of potassium ferricyanide solution (1 in 100) and ferric chloride solution (1 in 5). Dry the plate in a current of warm air for about 5 minutes or until a spot from *Standard solution B* is visible. Examine the chromatograms in ordinary light: the R_f value of the principal spot from the *Test solution* corresponds to that obtained from *Standard solution A*. No spot other than the principal spot obtained from the *Test solution* exceeds in size or intensity the principal spot obtained from *Standard solution B* (0.4%, corresponding to 0.2% of related compounds, the response factors for which are about double that of oxprenolol hydrochloride).

Assay—Dissolve about 450 mg of Oxprenolol Hydrochloride in 100 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass-calomel electrode system (with a salt bridge of a saturated solution of lithium chloride in glacial acetic acid). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 30.18 mg of $C_{15}H_{23}NO_3 \cdot HCl$.

Oxprenolol Hydrochloride Tablets

» Oxprenolol Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of oxprenolol hydrochloride ($C_{15}H_{23}NO_3 \cdot HCl$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards (11)—

USP Oxprenolol Hydrochloride RS

Identification—Transfer a portion of powdered Tablets, equivalent to about 100 mg of oxprenolol hydrochloride, to a suitable test tube containing 5 mL of water. Shake this mixture for about 1 minute and allow it to settle. Use the clear supernatant as the test solution. Prepare a Standard solution containing 20 mg of USP Oxprenolol Hydrochloride RS per mL. Apply separate 1- μ L portions of the test solution and of the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Line a suitable chromatographic chamber with filter paper, saturate the paper with the developing solvent consisting of a mixture of ethyl acetate, glacial acetic acid, and water (15:5:5), and allow to stand for about 30 minutes. Place the plate in the chamber, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 100° for 15 minutes. Spray the plate uniformly with a detection reagent consisting of a freshly prepared mixture of equal volumes of potassium ferricyanide solution (1 in 100) and ferric chloride solution (1 in 5). Dry the plate in a current of warm air for about 5 minutes. Examine the chromatograms in ordinary light: the R_f value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{15}H_{23}NO_3 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 272 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Oxprenolol Hydrochloride RS in the same medium.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{15}H_{23}NO_3 \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 80 mg of oxprenolol hydrochloride, to a 100-mL volumetric flask. Add 80 mL of a *Diluting solvent*, which consists of a mixture of methanol and 0.1 N hydrochloric acid (9:1), and shake by mechanical means for 1 hour. Dilute with *Diluting solvent* to volume, and mix. Filter, discarding the first 10 mL of the filtrate, transfer 25.0 mL of the clear filtrate to a 200-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix. Concomitantly determine the absorbances of this assay solution and a Standard solution of USP Oxprenolol Hydrochloride RS in the same solvent having a known concentration of about 0.1 mg per mL at the wavelength of maximum absorbance at about 274 nm and, in addition, at 300 nm, using *Diluting solvent* as the blank. Calculate the quantity, in mg, of

$C_{15}H_{23}NO_3 \cdot HCl$ in the portion of Tablets taken by the formula:

$$800C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Oxprenolol Hydrochloride RS in the Standard solution, and A_U and A_S are the differences between the absorbances at 274 nm and 300 nm of the assay solution and the Standard solution, respectively.

Oxprenolol Hydrochloride Extended-Release Tablets

» Oxprenolol Hydrochloride Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of oxprenolol hydrochloride ($C_{15}H_{23}NO_3 \cdot HCl$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards (11)—

USP Oxprenolol Hydrochloride RS

Identification—Tablets respond to the *Identification* test under *Oxprenolol Hydrochloride Tablets*.

Dissolution (711)—

Acid medium: 0.1 N hydrochloric acid; 900 mL.

Dissolution medium: simulated intestinal fluid TS (without enzyme); 900 mL.

Apparatus 1: 100 rpm.

Times: 1 hour in *Acid medium*; 1, 3, and 7 hours in *Dissolution medium*.

Procedure—Determine the amount of $C_{15}H_{23}NO_3 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorption at about 272 nm on the first solution under test, suitably diluted with *Acid medium*, in comparison with a Standard solution having a known concentration of USP Oxprenolol Hydrochloride RS in the same medium. Promptly transfer the basket containing the Tablet to *Dissolution medium*. After 1, 3, and 7 hours, respectively, remove 9.0 mL of the test solution and determine the amount of $C_{15}H_{23}NO_3 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorption at about 272 nm on the solution under test, suitably diluted with *Dissolution medium*, in comparison with a Standard solution having a known concentration of USP Oxprenolol Hydrochloride RS in the same medium. [NOTE—Replace the aliquots withdrawn for analysis with fresh portions of *Dissolution medium*.]

Tolerances—The percentages of the labeled amount of $C_{15}H_{23}NO_3 \cdot HCl$ dissolved at the times specified conform to *Acceptance Table 2*.

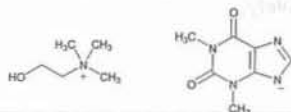
Time (hours)	Amount dissolved
1, in <i>Acid medium</i>	between 15% and 45%
1, in <i>Dissolution medium</i>	between 30% and 60%
3, in <i>Dissolution medium</i>	between 50% and 80%
7, in <i>Dissolution medium</i>	not less than 75%

Uniformity of dosage units (905): meet the requirements.

Procedure for content uniformity—Proceed as directed for *Procedure for the content uniformity* in the test for *Uniformity of dosage units* under *Oxprenolol Hydrochloride Tablets*.

Assay—Determine the mean value of the $C_{15}H_{23}NO_3 \cdot HCl$ contents of the Tablets as directed for *Uniformity of dosage units* under *Oxprenolol Hydrochloride Tablets*.

Oxtriphylline



$C_{12}H_{21}N_5O_3$ 283.33
 Ethanaminium, 2-hydroxy-N,N,N-trimethyl-, salt with 3,7-dihydro-1,3-dimethyl-1H-purine-2,6-dione;
 Choline salt with theophylline (1:1) [4499-40-5].

DEFINITION

Oxtriphylline contains NLT 61.7% and NMT 65.5% of anhydrous theophylline ($C_7H_8N_4O_2$), calculated on the dried basis.

IDENTIFICATION

- **A.**
Sample solution: 50 mg/mL in water
Analysis: To 10 mL of the *Sample solution* add 5 mL of mercuric-potassium iodide TS.
Acceptance criteria: A pale yellow precipitate is formed (presence of choline).
- **B.**
Sample solution: 50 mg/mL in water
Analysis: To 10 mL of the *Sample solution* add 5 drops of 6 N ammonium hydroxide and 5 mL of silver nitrate TS.
Acceptance criteria: A gelatinous precipitate is formed, and it coagulates on heating (presence of theophylline).

ASSAY

• THEOPHYLLINE

Sample solution: The solution retained in the test for *Choline Content*

Analysis: Add 35 mL of silver nitrate TS to the *Sample solution*, and swirl gently to promote complete precipitation. Titrate with 0.1 N sodium hydroxide VS to a green endpoint. Each mL of 0.1 N sodium hydroxide is equivalent to 18.02 mg of theophylline ($C_7H_8N_4O_2$).

Acceptance criteria: 61.7%–65.5% on the dried basis

OTHER COMPONENTS

• CHOLINE CONTENT

Indicator solution: Dissolve 30 mg of methyl red in 100 mL of methanol, and add 15 mL of methylene blue solution (1 in 1000).

Sample solution: 900 mg of Oxtriphylline in 50 mL of water

Analysis: Add 4 drops of *Indicator solution* to the *Sample solution*, and titrate with 0.1 N sulfuric acid VS to a purple endpoint. Each mL of 0.1 N sulfuric acid is equivalent to 12.12 mg of choline ($C_5H_{15}NO_2$). Retain the final solution for the *Assay for Theophylline*.

Acceptance criteria: The content of choline ($C_5H_{15}NO_2$) is between 652 and 693 mg/g of theophylline ($C_7H_8N_4O_2$) found in the *Assay*.

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.3%
- **CHLORIDE AND SULFATE**, *Chloride* (221)
Standard: 0.15 mL of 0.020 N hydrochloric acid
Sample: 0.50 g
Acceptance criteria: 0.02%; the *Sample* shows no more chloride than corresponds to the *Standard*.

• ORDINARY IMPURITIES (466)

Standard solution: Chloroform, alcohol, and formic acid (88:10:2)

Sample solution: Chloroform, alcohol, and formic acid (88:10:2)

Eluent: Chloroform, alcohol, and formic acid (88:10:2)

Visualization: 1

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE**, *Class I* (741):

185°–189°

- **LOSS ON DRYING** (731)

Analysis: Dry at 80° for 4 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS** (11)

USP Oxtriphylline RS

Oxtriphylline Oral Solution

DEFINITION

Oxtriphylline Oral Solution contains an amount of Oxtriphylline equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

IDENTIFICATION

- **A.** The retention time of the theophylline peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- **B.**

Sample solution: Place a volume of Oral Solution, equivalent to 100 mg of oxtriphylline, in a 60-mL separator containing 1 mL of glacial acetic acid and 40 mL of chloroform. Shake for 1 min, allow the phases to separate, and filter the lower phase through dry cotton into a 100-mL beaker.

Analysis: Transfer a portion of the *Sample solution*, equivalent to 10 mg of oxtriphylline, to a porcelain dish, and evaporate on a steam bath with the aid of a current of dry air to dryness. Add 1 mL of hydrochloric acid and 100 mg of potassium chlorate. Evaporate on a steam bath to dryness, and invert the dish over a vessel containing a few drops of 6 N ammonium hydroxide.

Acceptance criteria: The residue acquires a purple color.

ASSAY

• PROCEDURE

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 0.1 N potassium hydroxide to a pH of 5.8 ± 0.1 .

Mobile phase: Methanol and *Buffer* (1:4)

System suitability solution: 60 µg/mL of USP Oxtriphylline RS and 30 µg/mL of theobromine in water

Standard solution: 0.1 mg/mL of USP Oxtriphylline RS in water

Sample solution: Nominally 0.1 mg/mL of oxtriphylline, equivalent to 0.0636 mg/mL of theophylline, prepared by diluting Oral Solution with water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for theobromine and theophylline are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the theobromine and theophylline peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of theophylline from the *Sample solution*

r_S = peak response of theophylline from the *Standard solution*

C_S = concentration of USP Oxtriphylline RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of anhydrous theophylline, 180.17

M_{r2} = molecular weight of oxtriphylline, 283.33

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH** (791): 6.4–9.0

• **ALCOHOL DETERMINATION, Method I** (611) (if present): 90.0%–115.0% of the labeled amount, the labeled amount being NMT 20.0% of alcohol (C_2H_5OH)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label Oral Solution to state both the content of oxtriphylline and the content of anhydrous theophylline.
- **USP REFERENCE STANDARDS** (11)
USP Oxtriphylline RS

Oxtriphylline Tablets

DEFINITION

Oxtriphylline Tablets contain an amount of Oxtriphylline equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

IDENTIFICATION

- **A.** The retention time of the theophylline peak of the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the *Assay*.

ASSAY

• PROCEDURE

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 0.1 N potassium hydroxide to a pH of 5.8 ± 0.1 .

Mobile phase: Methanol and *Buffer* (1:4)

System suitability solution: 60 µg/mL of USP Oxtriphylline RS and 30 µg/mL of theobromine in water

Standard solution: 0.1 mg/mL of USP Oxtriphylline RS in water

Sample stock solution: Place 10 Tablets in a 1000-mL volumetric flask, and add 700 mL of water. Heat on a steam bath, with occasional shaking, until the Tablets have disintegrated. Cool to room temperature, dilute with water to volume, and filter.

Sample solution: Nominally 0.1 mg/mL of oxtriphylline, equivalent to 0.0636 mg/mL of theophylline, prepared by diluting the *Sample stock solution* with water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for theobromine and theophylline are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the theobromine and theophylline peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of theophylline from the *Sample solution*

r_S = peak response of theophylline from the *Standard solution*

C_S = concentration of USP Oxtriphylline RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of anhydrous theophylline, 180.17

M_{r2} = molecular weight of oxtriphylline, 283.33

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: Prepare a solution having a known concentration of USP Oxtriphylline RS in *Medium*.

Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium* if necessary

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 270 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) dissolved.

Tolerances: NLT 80% (Q) of the labeled amount of theophylline ($C_7H_8N_4O_2$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label Tablets to state both the content of oxtriphylline and the content of anhydrous theophylline.
- **USP REFERENCE STANDARDS** (11)
USP Oxtriphylline RS

Oxtriphylline Extended-Release Tablets

DEFINITION

Oxtriphylline Extended-Release Tablets contain an amount of Oxtriphylline equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous theophylline ($C_7H_8N_4O_2$).

IDENTIFICATION

- **A.** The retention time of the theophylline peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 10 mg/mL of USP Oxtriphylline RS in methanol

Sample solution: Nominally 10 mg/mL of oxtriphylline prepared as follows. Transfer a quantity of finely ground Tablets, equivalent to 100 mg of oxtriphylline, to a suitable test tube. Add 10 mL of methanol, shake on a vortex mixer for several min, and filter.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: Chloroform, alcohol, and formic acid (88:10:2)

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_f value to that of the *Standard solution*.

ASSAY

- **PROCEDURE**

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 0.1 N potassium hydroxide to a pH of 5.8 ± 0.1 .

Mobile phase: Methanol and *Buffer* (1:4)

System suitability solution: 60 μ g/mL of USP Oxtriphylline RS and 30 μ g/mL of theobromine in water

Standard solution: 0.1 mg/mL of USP Oxtriphylline RS in water

Sample stock solution: Place 10 Tablets in a 1000-mL volumetric flask. Add 700 mL of water, and heat on a steam bath, with occasional shaking, until the Tablets have disintegrated. Cool to room temperature, dilute with water to volume, and filter.

Sample solution: Nominally 0.1 mg/mL of oxtriphylline, equivalent to 0.0636 mg/mL of theophylline, prepared by diluting the *Sample stock solution* with water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for theobromine and theophylline are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the theobromine and theophylline peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline ($C_7H_8N_4O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of theophylline from the *Sample solution*

r_S = peak response of theophylline from the *Standard solution*

C_S = concentration of USP Oxtriphylline RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of theophylline in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of anhydrous theophylline, 180.17

M_{r2} = molecular weight of oxtriphylline, 283.33

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION (711)**

Test 1 (for products labeled as 400-mg Tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*. Proceed as directed for *Method B* under *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*, except use *Acceptance Table 2*.

Buffer: Transfer 27.22 g of monobasic potassium phosphate to a 4-L volumetric flask. Add 1 L of water and 816 mL of 0.2 N sodium hydroxide, and dilute with water to 3800 mL. Adjust with 0.2 N sodium hydroxide or phosphoric acid to a pH of 7.5, and dilute with water to volume.

Medium: 0.1 N hydrochloric acid for the first hour, then *Buffer*; 900 mL

Apparatus 2: 50 rpm

Times: 1, 3, 5, and 7 h

Standard solution: Prepare a solution having a known concentration of USP Oxtriphylline RS in *Medium*.

Sample solution: A filtered portion of the solution under test, diluted with *Medium* if necessary

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 248 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved
1	5%–30%
3	50%–70%
5	65%–85%
7	NLT 75%

Test 2 (for products labeled as 600-mg Tablets): If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*. Proceed as directed for *Method B* under *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*, except use *Acceptance Table 2*.

Buffer, Medium, Apparatus 2, Standard solution,

Sample solution, Instrumental conditions, and Analysis: Proceed as directed for *Test 1*.

Times: 1, 3, and 7 h

Tolerances: See *Table 2*.

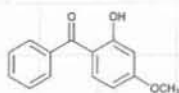
Table 2

Time (h)	Amount Dissolved
1	15%–40%
3	50%–70%
7	NLT 75%

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label the Tablets to state both the content of oxtriphylline and the content of anhydrous theophylline. The labeling indicates the *Dissolution* test with which the product complies.
- **USP REFERENCE STANDARDS (11)**
USP Oxtriphylline RS

OxybenzoneC₁₄H₁₂O₃

228.24

Methanone, (2-hydroxy-4-methoxyphenyl)phenyl-;
2-Hydroxy-4-methoxybenzophenone [131-57-7].

DEFINITION

Oxybenzone contains NLT 97.0% and NMT 103.0% of oxybenzone (C₁₄H₁₂O₃), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Mobile phase: Methanol and water (70:30)

Standard solution: 0.1 mg/mL of USP Oxybenzone RS in *Mobile phase*. Sonicate if necessary to dissolve.

Sample solution: 0.1 mg/mL of Oxybenzone in *Mobile phase*. Sonicate if necessary to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 289 nm

Column: 4.6-mm × 7.5-cm; 3.5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxybenzone (C₁₄H₁₂O₃) in the portion of Oxybenzone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxybenzone RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxybenzone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

SPECIFIC TESTS

- **CONGEALING TEMPERATURE (651):** NLT 62.0°

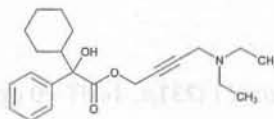
- **LOSS ON DRYING (731)**

Analysis: Dry a sample in a vacuum at 40° for 2 h.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**
USP Oxybenzone RS

Oxybutynin ChlorideC₂₂H₃₁NO₃ · HCl

393.95

Benzeneacetic acid, α-cyclohexyl-α-hydroxy-, 4-(diethylamino)-2-butynyl ester hydrochloride, (±)-;
4-(Diethylamino)-2-butynyl (±)-α-phenylcyclohexaneglycolate hydrochloride [1508-65-2].

DEFINITION

Oxybutynin Chloride contains NLT 97.0% and NMT 102.0% of oxybutynin chloride (C₂₂H₃₁NO₃ · HCl), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Buffer: A solution containing 6.67 g/L of monobasic potassium phosphate and 8.55 g/L of dibasic potassium phosphate

Mobile phase: Acetonitrile and *Buffer* (49:51)

Standard solution: 0.1 mg/mL of USP Oxybutynin Chloride RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Oxybutynin Chloride in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 7.5-cm; 3-μm or 3.5-μm packing L7

Column temperature: 45°

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxybutynin chloride (C₂₂H₃₁NO₃ · HCl) in the portion of Oxybutynin Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_s = concentration of USP Oxybutynin Chloride RS in the *Standard solution* (mg/mL)

C_u = concentration of Oxybutynin Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

OTHER COMPONENTS

• CHLORIDE CONTENT

Sample: 600 mg of Oxybutynin Chloride, previously dried

Analysis: Dissolve the *Sample* in 100 mL of water, and add 5 mL of nitric acid. Titrate (see *Titrimetry* (541)) with 0.1 N silver nitrate VS, using a platinum–silver chloride electrode system. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl).

Acceptance criteria: 8%–10%

IMPURITIES

• RESIDUE ON IGNITION (281): NMT 0.1%

Delete the following:

• HEAVY METALS, Method I (231): NMT 20 ppm (Official 1: Jan-2018)

• ORGANIC IMPURITIES

Buffer, Mobile phase, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability stock solution: 100 µg/mL each of USP Oxybutynin Related Compound B RS and USP Oxybutynin Related Compound C RS in *Mobile phase*

Standard stock solution: 1.0 mg/mL of USP Oxybutynin Chloride RS in *Mobile phase*

System suitability solution: Transfer 10.0 mL of the *System suitability stock solution* to a 100-mL volumetric flask, add 10.0 mL of *Standard stock solution*, and dilute with *Mobile phase* to volume.

Standard solution: 7.5 µg/mL of USP Oxybutynin Chloride RS from *Standard stock solution* in *Mobile phase*

Sample solution: 5.0 mg/mL of Oxybutynin Chloride in *Mobile phase*

System suitability

Sample: *System suitability solution*

[NOTE—See Table 1 for relative retention times.]

Suitability requirements

Resolution: NLT 1.1 between oxybutynin related compound B and oxybutynin related compound C

Relative standard deviation: NMT 2.0% for the oxybutynin peak

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms for a total time of NLT twice the retention time of the oxybutynin peak.

Calculate the percentage of each impurity in the portion of Oxybutynin Chloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

r_u = peak response of each impurity from the *Sample solution*

r_s = peak response of oxybutynin from the *Standard solution*

C_s = concentration of USP Oxybutynin Chloride RS in the *Standard solution* (mg/mL)

C_u = concentration of Oxybutynin Chloride in the *Sample solution* (mg/mL)

F = relative response factor for each impurity (see Table 1)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxybutynin related compound A ^a	0.08	1.4	0.5
Diphenyl analog of oxybutynin chloride ^b	0.37	2.7	0.1
Oxybutynin related compound B ^c	0.65	1.3	1.0
Oxybutynin related compound C ^d	0.79	1.0	1.0
Oxybutynin	1.0	—	—
Cyclohexenyl analog of oxybutynin chloride ^e	1.8	0.4	1.0
Ethylpropyl analog of oxybutynin chloride ^f	1.9	1.0	0.1
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	1.0

^a Phenylcyclohexylglycolic acid (cyclohexylmandelic acid, or CHMA).

^b 4-(Diethylamino)but-2-ynyl 2-hydroxy-2,2-diphenylacetate.

^c Methyl ester of phenylcyclohexylglycolic acid (methyl ester of cyclohexylmandelic acid, or CHMME).

^d Methylethyl analog of oxybutynin chloride (4-(ethylmethylamino) but-2-ynyl (±)-2-cyclohexyl-2-hydroxy-2-phenylacetate).

^e 4-(Diethylamino)but-2-ynyl (±)-2-(cyclohex-3-enyl)-2-cyclohexyl-2-hydroxyacetate.

^f 4-(Ethylpropylamino)but-2-ynyl (±)-2-cyclohexyl-2-hydroxy-2-phenylacetate.

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry a sample at 105° for 2 h.

Acceptance criteria: NMT 3%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• USP REFERENCE STANDARDS (11)

USP Oxybutynin Chloride RS

USP Oxybutynin Related Compound B RS

Methyl ester of phenylcyclohexylglycolic acid, or CHMME (cyclohexyl mandelic acid methyl ester).

USP Oxybutynin Related Compound C RS

Methylethyl analog of oxybutynin chloride, or 4-(ethylmethylamino) but-2-ynyl (±) 2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride.

Oxybutynin Chloride Oral Solution

» Oxybutynin Chloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{22}H_{31}NO_3 \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Oxybutynin Chloride RS

Identification—Place a volume of Oral Solution, equivalent to about 50 mg of oxybutynin chloride, in a separator, and extract with 10 mL of chloroform. The extract so obtained responds to the *Thin-Layer Chromatographic Identification Test* (201), methanol being used as the developing solvent, and iodine vapor being used to visualize the spots.

Assay—

pH 4 Phosphate buffer—Place 38 mL of 0.2 M dibasic sodium phosphate in a 100-mL volumetric flask. Dilute with 0.1 M citric acid to volume, and mix. Adjust the pH, if necessary, with either the dibasic sodium phosphate solution or the citric acid solution.

pH 5.6 Phosphate buffer—Place 58 mL of 0.2 M dibasic sodium phosphate in a 100-mL volumetric flask. Dilute with 0.1 M citric acid to volume, and mix. Adjust the pH, if necessary, with either the dibasic sodium phosphate solution or the citric acid solution.

Bromocresol green solution—Transfer 125 mg of bromocresol green to a 25-mL volumetric flask, dissolve in 3.5 mL of 0.05 N sodium hydroxide, dilute with water to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Oxybutynin Chloride RS in 0.05 N sulfuric acid to obtain a solution having a known concentration of about 100 µg per mL.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 10 mg of oxybutynin chloride, to a 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Separately transfer 10.0 mL of the *Standard preparation* and the *Assay preparation* to separate 125-mL separators. Add 20 mL of pH 4 Phosphate buffer to each separator, and extract each solution with a 25-mL portion of chloroform. [NOTE—Allow at least 10 minutes for the layers to separate.] Collect the chloroform extracts in respective 125-mL separators, each containing a mixture of 2 mL of pH 5.6 Phosphate buffer and 1 mL of Bromocresol green solution. Shake the separators, and filter the chloroform extracts through rayon pledgets, collecting the extracts in respective 100-mL volumetric flasks. Repeat the double extractions with 25-mL portions of chloroform. Wash the rayon pledgets with chloroform, collecting the washings in the respective 100-mL volumetric flasks. Dilute both solutions with chloroform to volume, and mix. Concomitantly determine the absorbances of both solutions at the wavelength of maximum absorbance at about 415 nm, with a suitable spectrophotometer, against a blank prepared using 10 mL of 0.05 N sulfuric acid treated in the same manner as the *Standard preparation* and the *Assay preparation*. Calculate the quantity, in mg, of $C_{22}H_{31}NO_3 \cdot HCl$ in each mL of Oral Solution taken by the formula:

$$(0.1C / V)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Oxybutynin Chloride RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Oxybutynin Chloride Tablets

DEFINITION

Oxybutynin Chloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of $C_{22}H_{31}NO_3 \cdot HCl$.

IDENTIFICATION

- **THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

Sample solution: Add a portion of powdered Tablets, equivalent to about 50 mg of oxybutynin chloride, to 10 mL of chloroform. Mix for two minutes, and centrifuge. Use the supernatant layer.

Developing solvent system: Methanol

Visualization: Iodine vapor

ASSAY

- **PROCEDURE**

Solution A: Methanol, water, and triethylamine (800:3200:0.9). Adjust with phosphoric acid to a pH of 3.5 ± 0.05 .

Mobile phase: Acetonitrile and Solution A (1:4)

Standard solution: 0.05 mg/mL of USP Oxybutynin Chloride RS in *Mobile phase*

Sample solution: Transfer an amount of powdered Tablets (from NLT 20 Tablets) nominally equivalent to 50 mg of oxybutynin chloride to a 1000-mL volumetric flask. Add about 400 mL of *Mobile phase*, sonicate for about 10 min, shake by mechanical means for about 45 min, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 203 nm

Column: 4-mm × 30-cm; packing L10

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{22}H_{31}NO_3 \cdot HCl$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxybutynin Chloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION (711)**

Test 1

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter. Dilute with *Medium* if necessary.

Analysis: Determine the amount of $C_{22}H_{31}NO_3 \cdot HCl$ dissolved using the method set forth in the *Assay*, making any necessary modifications to the concentration of the *Standard solution* to correspond to that of the solution under test and injecting 100 µL of both solutions.

Tolerances: NLT 80% (Q) of the labeled amount of $C_{22}H_{31}NO_3 \cdot HCl$ is dissolved.

Test 2: If the products complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: 5 µg/mL of USP Oxybutynin Chloride RS in *Medium*. This solution is stable for 5 days at ambient conditions.

Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter, discarding the first few mL.

Mobile phase: Water, acetonitrile, and phosphoric acid (760:240:1)

Chromatographic system(See *Chromatography* ⟨621⟩, *System Suitability*.)**Mode:** LC**Detector:** UV 203 nm**Column:** 4.6-mm × 7.5-cm; 3.5-μm packing L7**Column temperature:** 40°**Flow rate:** 1.5 mL/min**Injection size:** 100 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 3.0%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of oxybutynin chloride dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of oxybutynin chloride in the *Standard solution* L = Tablet label claim (mg) V = volume of *Medium* (mL), 900**Tolerances:** NLT 80% (Q) of the labeled amount of oxybutynin chloride is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Oxybutynin Chloride RS

Oxybutynin Chloride Extended-Release Tablets

DEFINITIONOxybutynin Chloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$).**IDENTIFICATION**

- **A. INFRARED ABSORPTION** ⟨197⟩

Standard: Dissolve 15 mg of USP Oxybutynin Chloride RS in 5 mL of water. Adjust with 0.1 N sodium hydroxide to a pH of between 7 and 8. Extract the solution twice with 10 mL of ether. Combine the extracts, evaporate the ether, and dry under vacuum over silica gel for at least 30 min. Redissolve the dried residue in a small amount of acetone, transfer the solution to an IR salt plate, and evaporate to cast a thin film.

Sample: Add a quantity of finely powdered Tablets, equivalent to about 15 mg of oxybutynin chloride, to 5 mL of water per Tablet. Mix for 1 min. Adjust with 0.1 N sodium hydroxide to a pH between 7 and 8. Extract the solution twice with 10 mL of ether. Combine the extracts, evaporate the ether, and dry under vacuum over silica gel for at least 30 min. Redissolve the dried residue in a small amount of acetone, transfer the solution to an IR salt plate, and evaporate to cast a thin film.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE****Diluent:** Use water adjusted with phosphoric acid to a pH of 3.5.**Solution A:** Methanol and acetonitrile (1:1)**Mobile phase:** Acetonitrile, triethylamine, and water (700:3:1300). Adjust with phosphoric acid to a pH of 3.9.**Impurity stock solution:** 0.11 mg/mL of USP Oxybutynin Related Compound A RS in acetonitrile**Standard stock solution:** 0.37 mg/mL of USP Oxybutynin Chloride RS in acetonitrile**System suitability solution:** Transfer 10 mL of the *Standard stock solution* and 1 mL of the *Impurity stock solution* to a 100-mL volumetric flask, and dilute with *Diluent* to volume.**Standard solution:** 0.1 mg/mL in *Diluent* from the *Standard stock solution***Sample solution**

For Tablets that contain 5 mg of oxybutynin chloride: Place 10 Tablets in a 500-mL volumetric flask, add 150 mL of *Solution A*, and stir for at least 4 h or until dissolved. Dilute with *Diluent* to volume. Mix thoroughly, centrifuge, and use the clear supernatant.

For Tablets that contain 10 mg or more of oxybutynin chloride: Place 10 Tablets in a 1000-mL volumetric flask, add 300 mL of *Solution A*, and stir for at least 4 h or until dissolved. Dilute with *Diluent* to volume. If necessary, make a further dilution with *Diluent* to obtain a solution having a final concentration equivalent to 0.1 mg/mL of oxybutynin chloride. Mix thoroughly, centrifuge, and use the clear supernatant.

Chromatographic system(See *Chromatography* ⟨621⟩, *System Suitability*.)**Mode:** LC**Detector:** UV 220 nm**Column:** 4.6-mm × 15-cm; packing L11**Flow rate:** 1.5 mL/min**Injection size:** 50 μL**System suitability****Sample:** *System suitability solution*

[NOTE—The relative retention times for oxybutynin and oxybutynin related compound A are about 1.0 and 1.6, respectively.]

Suitability requirements**Resolution:** NLT 1.5 between oxybutynin and oxybutynin related compound A**Tailing factor:** Greater than 0.75 and NMT 2.5 for each peak**Relative standard deviation:** NMT 3% for each compound for six replicate injections**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Oxybutynin Chloride RS in the *Standard solution* (mg/mL) C_U = nominal concentration of oxybutynin chloride in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**

- **DISSOLUTION** ⟨711⟩

Test 1**Medium:** Simulated gastric fluid without enzyme; 50 mL**Apparatus 7:** See *Drug Release* ⟨724⟩, 30 cycles/min; 2- to 3-cm amplitude, at $37.0 \pm 0.5^\circ$

Times: 4, 10, and 24 h

Solution A: 4.83 g/L of monobasic sodium phosphate in water. Add 2.3 mL/L of triethylamine, and adjust with phosphoric acid to a pH of 2.2 ± 0.2 .

Mobile phase: Acetonitrile and *Solution A* (7:13)

Solution B: To 1 L of water add phosphoric acid dropwise to a pH of 3.5, and mix well.

Standard stock solutions: 250, 300, and 350 µg/mL

each of USP Oxybutynin Chloride RS in acetonitrile
Standard solutions: Prepare a series of dilutions of the *Standard stock solutions* in *Solution B* having final concentrations similar to those expected in the *Sample solution*.

System suitability solution: Use a medium range *Standard solution* of USP Oxybutynin Chloride RS.

Sample solution: Use portions of the solution under test. If the solution is cloudy, centrifuge at 2000 rpm for 10 min, and use the supernatant.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 5-cm; packing L11

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection size: 50 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Tailing factor: Greater than 0.5 and less than 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solutions* and *Sample solution*

Construct a calibration curve by plotting the peak response versus concentration of the *Standard solutions*. A weighing factor, $1/x$, is applied to the regression line of the calibration curve to enhance the accuracy of the low standard concentrations. Determine the percentage of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved in each interval from a linear regression analysis of the calibration curve.

Tolerances: See *Tables 1* and *2*.

Table 1. For Tablets labeled to contain 5 or 10 mg of oxybutynin chloride

Time (h)	Amount Dissolved
4	NMT 20%
10	34.5%–59.5%
24	NLT 80%

Table 2. For Tablets labeled to contain 15 mg of oxybutynin chloride

Time (h)	Amount Dissolved
4	NMT 20%
10	34.5%–59.5%
24	NLT 75%

The percentages of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* <711>.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Acid stage medium: Simulated gastric fluid, without enzymes, pH 1.2 ± 0.05 ; 250 mL (first row)

Buffer stage medium: Simulated intestinal fluid, without enzymes, pH 6.8 ± 0.1 ; 250 mL (rows 2–4)

Apparatus 3: 25 dips/min; 20-mesh polypropylene screen on top and bottom; 30 s drip time

Times: 2 h in the *Acid stage medium* (first row); 4, 8, and 16 h (corresponding to 2, 6, and 14 h after changing the medium) in the *Buffer stage medium* (rows 2–4)

Solution A: Transfer 1 mL of triethylamine to 1000 mL of water. Adjust with phosphoric acid to a pH of 3.50 ± 0.05 .

Mobile phase: Acetonitrile and *Solution A* (4:1)

Standard stock solution: 0.2 mg/mL of USP Oxybutynin Chloride RS in *Acid stage medium*

Working standard solution: Transfer 5.0 mL of the *Standard stock solution* for Tablets labeled to contain 5 mg, transfer 10 mL for Tablets labeled to contain 10 mg, or transfer 15 mL for Tablets labeled to contain 15 mg to a 100-mL volumetric flask. Dilute with *Buffer stage medium* to volume.

Sample solution: Centrifuge a portion of the solution under test at approximately 3000 rpm for 10 min. Use the supernatant.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 203 nm

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 1.5 mL/min

Injection size: 25 µL

System suitability

Sample: *Working standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Working standard solution* and *Sample solution*

Calculate the percentage of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at each time point (C_{T2} , C_{T4} , C_{T8} , C_{T16}):

$$C_i = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Working standard solution*

C_S = concentration of the *Working standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 250 mL

C_{T2} = percentage dissolved at 2 h, C_2

C_{T4} = percentage dissolved at 4 h, $C_2 + C_4$

C_{T8} = percentage dissolved at 8 h, $C_2 + C_4 + C_8$

C_{T16} = percentage dissolved at 16 h, $C_2 + C_4 + C_8 + C_{16}$

Tolerances: See *Tables 3* and *4*.

Table 3. For Tablets labeled to contain 5 or 10 mg

Time (h)	Amount Dissolved
2	0%–10%
4	10%–30%
8	40%–65%
16	NLT 80%

Table 4. For Tablets labeled to contain 15 mg

Time (h)	Amount Dissolved
2	0%–10%
4	10%–30%
8	35%–65%
16	NLT 75%

The percentages of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* (711).

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution* Test 3.

Medium: Simulated gastric fluid without enzyme; 50 mL

Apparatus 7: See *Drug Release* (724). Use acrylic rods. 30 dips/min, $37.0 \pm 0.5^\circ$, 10 s drip time. Dip time interval: row 1, 1 h; row 2, 3 h; row 3, 6 h; row 4, 5 h; row 5, 9 h.

Times: 4, 10, and 24 h

pH 2.3 phosphate buffer: 3.4 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid or 2 N potassium hydroxide to a pH of 2.30 ± 0.05 .

Standard solution: ($L/200$) mg/mL of USP Oxybutynin Chloride RS in *Medium*, where L is the Tablet label claim, in mg

Sample solution: Pass a portion of the solution under test through a suitable nylon filter of 0.45- μ m pore size, discarding the first few mL.

Mobile phase: pH 2.3 phosphate buffer and acetonitrile (7:3)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 15-cm; packing L10

Flow rate: 1.0 mL/min

Injection size: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount, in mg, of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at each time interval:

$$\text{Result} = (r_u/r_s) \times (C_s/L) \times V$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 50 mL

Calculate the percentage of oxybutynin dissolved:

$$\text{Result} = \Sigma(\text{amount dissolved at current time interval} + \text{amount dissolved at previous time intervals}) \times 100/L$$

Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved
4	NMT 25%
10	40%–65%
24	NLT 75%

The percentages of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* (711).

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution* Test 4.

Acid stage medium: 0.1 N hydrochloric acid; 900 mL

Buffer stage medium: pH 6.0 sodium phosphate buffer with 0.2% of sodium lauryl sulfate; 900 mL

Apparatus 2: 50 rpm, with sinkers. [NOTE—A suitable sinker is available as catalog number CAPWHT-2S from www.QLA-LLC.com.]

Times: 2 h in the *Acid stage medium*; 4, 6, and 14 h (corresponding to 2, 4, 12 h after changing the medium) in the *Buffer stage medium*

Standard solution: ($L/1000$) mg/mL of USP Oxybutynin Chloride RS in *Buffer stage medium*, where L is the Tablet label claim, in mg

Sample solution: Pass a portion of the solution under test through a suitable PVDF filter of 0.45- μ m pore size.

pH 3.5 phosphate buffer: 6.94 mg/mL of monobasic potassium phosphate in water. Adjust with diluted phosphoric acid to a pH of 3.50 ± 0.05 .

Mobile phase: pH 3.5 phosphate buffer and acetonitrile (1:1)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 15-cm; packing L7

Flow rate: 1.0 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (mg/mL) of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) (C_i) at each time point:

$$C_i = (r_u/r_s) \times C_s$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of the *Standard solution* (mg/mL)

Calculate the cumulative percentage of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved (Q_i) at each time point (i):

At $i = 1$

$$Q_1 = (C_1 \times V/L) \times 100$$

At $i = 2$ to n

$$\frac{(C_1 \times 900) + \sum_{j=2}^{n-1} C_j V_s + C_n \times [900 - (n-2)V_s] \times 100}{L}$$

$i = 1, 2, \dots, n$

$j = 2, 3, \dots, n-1$

V_s = sampling volume (mL)

C_i = concentration of oxybutynin chloride in the *Sample solution* at time point i (mg/mL)

C_j = concentration of oxybutynin chloride in the *Sample solution* at time point 2 through $n-1$ (mg/mL)

L = label claim (mg/Tablet)

Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Dissolved
2	NMT 10%
4	10%–40%

Table 6 (Continued)

Time (h)	Amount Dissolved
6	40%–75%
14	NLT 85%

The percentages of the labeled amount of oxybutynin chloride ($C_{22}H_{31}NO_3 \cdot HCl$) dissolved at the times specified conform to Acceptance Table 2 in Dissolution (711).

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Diluent, Solution A, Mobile phase, Impurity stock solution, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Impurity standard solution: 1 µg/mL of USP Oxybutynin Related Compound A RS in Diluent from the Impurity stock solution

Analysis

Samples: Impurity standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each impurity from the Sample solution

r_S = peak response from the Impurity standard solution

C_S = concentration of USP Oxybutynin Related Compound A RS in the Standard solution (mg/mL)

C_U = concentration of the Sample solution (mg/mL)
[NOTE—Disregard any peak less than 0.1%.]

Acceptance criteria

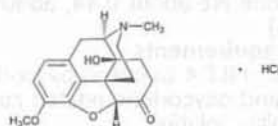
Individual impurities: NMT 1% of oxybutynin related compound A is found.

Total impurities: NMT 2%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one Dissolution test is given, the labeling states the Dissolution test used only if Test 1 is not used.
- **USP REFERENCE STANDARDS (11)**
USP Oxybutynin Chloride RS
USP Oxybutynin Related Compound A RS
Phenylcyclohexylglycolic acid.
 $C_{14}H_{18}O_3$ 234.30

Oxycodone Hydrochloride



$C_{18}H_{21}NO_4 \cdot HCl$ 351.82
Morphinan-6-one, 4,5-epoxy-14-hydroxy-3-methoxy-17-methyl-, hydrochloride, (5α)-;
4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one hydrochloride [124-90-3].

DEFINITION

Oxycodone Hydrochloride contains NLT 97.0% and NMT 103.0% of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$), calculated on the anhydrous, solvent-free basis.

IDENTIFICATION

• A. PROCEDURE

Sample solution: Dissolve 250 mg in 25 mL of water.

Analysis: Render the 25 mL of Sample solution alkaline with 6 N ammonium hydroxide. Allow the mixture to stand until a precipitate is formed. Filter, wash the precipitate with 50 mL of cold water, and dry at 105° for 2 h.

Acceptance criteria: The precipitate melts between 218° and 223°, but the range between the beginning and the end of melting does not exceed 2° (see Melting Range or Temperature (741)).

- **B. INFRARED ABSORPTION (197K):** Use a portion of the dried precipitate obtained in Identification test A.
- **C.** The retention time of the oxycodone peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

ASSAY

• PROCEDURE

Mobile phase: 0.005 M sodium 1-hexanesulfonate, methanol, triethylamine, and phosphoric acid (900:100:2:5). Adjust with 50% sodium hydroxide solution to a pH of 2.5 ± 0.1 and filter.

System suitability solution: 13 µg/mL of codeine phosphate and 9 µg/mL of oxycodone in Mobile phase

Standard solution: 0.9 mg/mL of USP Oxycodone RS in Mobile phase

Sample solution: 1 mg/mL of Oxycodone Hydrochloride in Mobile phase. [NOTE—Pass a portion of this solution through a filter of 0.5-µm or finer pore size, and use the filtrate as the Sample solution.]

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 206 nm

Column: 3.9-mm × 15-cm; 4-µm packing L7

Column temperature: 50°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: NLT 2 times the retention time of oxycodone

System suitability

Samples: System suitability solution and Standard solution

[NOTE—The relative retention times for codeine and oxycodone are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between codeine and oxycodone, System suitability solution

Tailing factor: 0.75–1.25, Standard solution

Relative standard deviation: NMT 2.0% from replicate injections, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Oxycodone RS in the Standard solution (mg/mL)

C_U = concentration of Oxycodone Hydrochloride in the Sample solution (mg/mL)

M_{r1} = molecular weight of oxycodone hydrochloride, 351.82

M_{r2} = molecular weight of oxycodone base, 315.37

Acceptance criteria: 97.0%–103.0% on the anhydrous, solvent-free basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.05%. [NOTE—Use of sulfuric acid is omitted.]

- **LIMIT OF ALCOHOL**

Internal standard stock solution: Transfer 6.0 mL of isopropyl alcohol to a 500-mL volumetric flask, and dilute with water to volume. [NOTE—The isopropyl alcohol must be free of alcohol impurities.]

Internal standard solution: Transfer 5.0 mL of the *Internal standard stock solution* to a 100-mL volumetric flask, and dilute with water to volume.

Standard stock solution: 16 mg/mL of alcohol (C_2H_5OH) in water

Standard solution: Pipet 3.0 mL of the *Standard stock solution* and 5.0 mL of the *Internal standard stock solution* into a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer about 240 mg of Oxycodone Hydrochloride to a 15-mL centrifuge tube, add 5.0 mL of the *Internal standard solution*, and mix to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 4-mm \times 1.8-m glass; packed with 80- to 100-mesh support S3

Carrier gas: Helium

Temperatures

Injection port: 170°

Column: 150°. [NOTE—Condition the column overnight at 235° with a slow flow of carrier gas.]

Detector: 170°

Injection volume: 5 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2 between isopropyl alcohol and alcohol

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of alcohol (C_2H_5OH) in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the alcohol peak to the isopropyl alcohol from the *Sample solution*

R_S = peak response ratio of the alcohol peak to the isopropyl alcohol from the *Standard solution*

C_S = concentration of alcohol in the *Standard solution* (mg/mL)

C_U = concentration of Oxycodone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

- **ORGANIC IMPURITIES**

[NOTE—On the basis of the synthetic route, perform either (a) *Procedure 1* and *Procedure 2* or (b) *Procedure 3*. *Procedure 1* and *Procedure 2* are recommended if 8 β -hydroxyoxycodone (7,8-dihydro-8 β -14-dihydroxycodone) is a potential impurity.]

Procedure 1

Analysis: Use the chromatogram of the *Sample solution* from the *Assay* to calculate the percentage of each impurity in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity

r_T = sum of the responses of all the peaks

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxymorphone	0.31	0.15
Noroxymorphone ^a	0.33	0.15
10-Hydroxyoxycodone ^b	0.53	0.15
6- α -Oxycodol ^c	0.67	0.25
8 β -Hydroxyoxycodone (7,8-dihydro-8 β -14-dihydroxycodone) ^d	0.71	0.15
Hydrocodone	1.19	0.15
Individual unspecified impurity	—	0.10
Total impurities	—	2.0

^a 4,5 α -Epoxy-3,14-dihydroxymorphinan-6-one.

^b 4,5 α -Epoxy-10 α ,14-dihydroxy-3-methoxy-17-methylmorphinan-6-one.

^c 4,5 α -Epoxy-3-methoxy-17-methylmorphinan-6 α ,14-diol.

^d 4,5 α -Epoxy-8 β ,14-dihydroxy-3-methoxy-17-methylmorphinan-6-one.

Procedure 2: Limit of Oxycodone Related Compound A (14-Hydroxycodone) and Oxycodone Related Compound C (Codeinone)

Solution A: Dissolve 3.45 g of monobasic sodium phosphate in 1000 mL of water. Add 5.41 g of sodium dodecyl sulfate and mix. Filter and adjust with 50% (w/v) sodium hydroxide solution to a pH of 7.50 \pm 0.05.

Solution B: Water and phosphoric acid (9:1)

Mobile phase: Prepare a mixture of acetonitrile, methanol, and *Solution A* (15.8: 12.0: 72.2), and adjust with *Solution B* to a pH of 7.80 \pm 0.01.

Diluent: Prepare a mixture of water and *Solution B* (9:1).

Unspiked oxycodone hydrochloride solution: 50 mg/mL of USP Oxycodone Hydrochloride RS in *Diluent*

System suitability solution: 100 μ g/mL of USP Oxycodone Hydrochloride RS and 5 μ g/mL each of USP Oxycodone Related Compound A RS and USP Oxycodone Related Compound C RS in *Diluent*

Standard solution: 50 mg/mL of USP Oxycodone Hydrochloride RS and 0.5 μ g/mL each of USP Oxycodone Related Compound A RS and USP Oxycodone Related Compound C RS in *Diluent*

Sample solution: 50 mg/mL of Oxycodone Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.0-mm \times 15-cm; 3.5- μ m packing L1

Column temperature: 40°

Flow rate: 0.7 mL/min

Injection volume: 5 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for oxycodone related compound C, oxycodone related compound A, and oxycodone are about 0.44, about 0.85, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4 between oxycodone related compound A and oxycodone related compound C, *System suitability solution*

Tailing factor: NMT 2.0, *System suitability solution*

Relative standard deviation: NMT 20% for oxycodone related compound A and oxycodone related compound C, *Standard solution*

Analysis

Samples: *Diluent*, *Unspiked oxycodone hydrochloride solution*, *Standard solution*, and *Sample solution*

Calculate the percentage of oxycodone related compound A and oxycodone related compound C in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxycodone related compound A or oxycodone related compound C from the *Sample solution*

r_S = peak response of oxycodone related compound A or oxycodone related compound C minus the response of the *Unspiked oxycodone hydrochloride solution* from the *Standard solution*

C_S = concentration of USP Oxycodone Related Compound A RS or USP Oxycodone Related Compound C RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxycodone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxycodone related compound C ^a	0.44	0.001
Oxycodone related compound A ^b	0.85	0.001
Oxycodone	1.0	—

^a Codeinone (C₁₈H₁₉NO₃).

^b 14-Hydroxycodeinone (C₁₈H₂₁NO₄).

(Procedure 2 postponed indefinitely)

Procedure 3

Buffer: Mix 4.0 mL of heptafluorobutyric acid with 2000 mL of water and adjust with ammonium hydroxide to a pH of 2.3 ± 0.1.

Solution A: Methanol and Buffer (23:77)

Solution B: Methanol, tetrahydrofuran, and Buffer (20:3:77)

Mobile phase: See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2	100	0
30	0	100
55	0	100
55.1	100	0
65	100	0

Diluent: Mix 3.0 mL of trifluoroacetic acid with 1000 mL of water.

System suitability solution: 0.0067 mg/mL each of USP Hydrocodone RS and USP Oxycodone Related Compound A RS, and 3.0 mg/mL of USP Oxycodone Hydrochloride RS in *Diluent*

Standard solution: 0.0067 mg/mL of USP Hydrocodone RS in *Diluent*

Sample solution: 3.0 mg/mL of Oxycodone Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 3-μm packing L1

Column temperature: 38°

Flow rate: 0.8 mL/min

Injection volume: 50 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between oxycodone and hydrocodone; NLT 1.0 between hydrocodone and oxycodone related compound A, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oxycodone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of hydrocodone from the *Standard solution*

C_S = concentration of USP Hydrocodone RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxycodone Hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of hydrocodone hydrochloride, 335.83

M_{r2} = molecular weight of hydrocodone, 299.36

F = relative response factor (see Table 4)

Acceptance criteria: See Table 4. Disregard any peaks below 0.03%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxymorphone hydrochloride	0.54	0.93	0.15
1-Hydroxyoxycodone hydrochloride ^a	0.69	1.00	0.15
6-Oxycodol hydrochloride ^b	0.79	1.16	0.25
Oxycodone hydrochloride	1.00	—	—
Hydrocodone hydrochloride	1.14	1.00	0.50
14-Hydroxycodeinone hydrochloride (oxycodone related compound A hydrochloride) ^c	1.18	0.99	0.25
Noroxycodone hydrochloride ^d	1.26	0.94	0.50

^a 4,5α-Epoxy-1,14-dihydroxy-3-methoxy-17-methylmorphinan-6-one hydrochloride.

^b 4,5α-Epoxy-3-methoxy-17-methylmorphinan-6,14-diol hydrochloride.

^c 4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-7-ene-6-one (oxycodone related compound A hydrochloride salt).

^d 4,5α-Epoxy-3-methoxy-17-methylmorphinan-6-one hydrochloride.

Table 4 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Individual unspecified impurity	—	—	0.10
Total impurities	—	—	1.5

^a 4,5 α -Epoxy-1,14-dihydroxy-3-methoxy-17-methylmorphinan-6-one hydrochloride.

^b 4,5 α -Epoxy-3-methoxy-17-methylmorphinan-6,14-diol hydrochloride.

^c 4,5 α -Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-7-ene-6-one (oxycodone related compound A hydrochloride salt).

^d 4,5 α -Epoxy-3-methoxy-17-methylmorphinan-6-one hydrochloride.

SPECIFIC TESTS

• CONTENT OF CHLORIDE

Sample solution: 6 mg/mL in methanol

Analysis: To 50 mL of the *Sample solution*, add 5 mL of glacial acetic acid and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.

Acceptance criteria: 9.8%–10.4% on the anhydrous, solvent-free basis

• OPTICAL ROTATION (7815), *Specific Rotation*

Sample solution: 25 mg/mL of Oxycodone Hydrochloride in water on the anhydrous, solvent-free basis

Acceptance criteria: -137° to -149°

• WATER DETERMINATION (921), *Method I*: NMT 7.0%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in tight containers.

• LABELING: The label states with which *Organic Impurities* procedure the article complies if *Organic Impurities, Procedure 1* is not used.

• USP REFERENCE STANDARDS (11)

USP Hydrocodone RS

USP Oxycodone RS

USP Oxycodone Hydrochloride RS

USP Oxycodone Related Compound A RS

Also known as 14-Hydroxycodeinone;

4,5 α -Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-7-ene-6-one.

$C_{18}H_{19}NO_4$ 313.35

USP Oxycodone Related Compound C RS

Also known as Codeinone;

4,5 α -Epoxy-3-methoxy-17-methylmorphinan-7-ene-6-one.

$C_{18}H_{19}NO_3$ 297.35

Oxycodone Hydrochloride Oral Solution

DEFINITION

Oxycodone Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$).

IDENTIFICATION

• A.

Sample solution: Transfer an amount equivalent to 15 mg of oxycodone from Oral Solution to a separatory funnel, add 10 mL of 0.01 N hydrochloric acid, and extract with four 40-mL portions of chloroform, collecting the chloroform extracts in a second separator. Wash the combined chloroform extracts with 5 mL of 0.01 N hydrochloric acid, and discard the chloroform layer. Combine the acidic wash with the aqueous solution remaining in the first separator and adjust with 6 N ammonium hydroxide to a pH of 9.5 ± 0.5 . Extract with one 50-mL and two 20-mL portions of chloroform,

and filter the chloroform extracts through chloroform-washed cotton, collecting the filtrate in a 100-mL volumetric flask. Dilute with water-saturated chloroform to volume, and mix.

Standard solution: Prepare a solution using 12 mg of USP Oxycodone RS and 25 mL of 0.01 N hydrochloric acid, and proceed as directed above, beginning with "extract with four 40-mL portions of chloroform".

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*, concomitantly measured.

• B. THIN-LAYER CHROMATOGRAPHY

Standard solution: Evaporate 5 mL of the *Standard solution* obtained from *Identification* test A just to dryness. Dissolve the residue in 1.0 mL of chloroform.

Sample solution: Evaporate 5 mL of the *Sample solution* obtained from *Identification* test A just to dryness. Dissolve the residue in 1.0 mL of chloroform.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 μ L

Developing solvent system: Acetone, toluene, ether, and ammonium hydroxide (6:4:1:0.3)

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the plate until the solvent front has moved about three-fourths of the length of the plate, remove it, mark the solvent front, allow the solvent to evaporate, and spray with iodoplatinate TS.

Acceptance criteria: The principal spot from the *Sample solution* corresponds in color, size, and R_f value to that from the solution from the *Standard solution*, and no other spots are observed.

• C. The retention time of the oxycodone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile, 0.01 M sodium 1-hexanesulfonate, and glacial acetic acid (25:74:1). Adjust with 5 N sodium hydroxide to a pH of 3.5.

Standard solution: 0.045 mg/mL of USP Oxycodone RS in *Mobile phase*

Sample solution: Transfer an amount equivalent to 5 mg of oxycodone hydrochloride from Oral Solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this mixture through a filter having a 0.5- μ m or finer pore size, and use the clear filtrate as the *Sample solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1.7 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of oxycodone hydrochloride in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of oxycodone hydrochloride, 351.82
 M_{r2} = molecular weight of oxycodone base, 315.37
 Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

- **ALCOHOL DETERMINATION, Method II (611):** If present, 85.0%–115.0% of the labeled amount of alcohol (C_2H_5OH), determined by the gas-liquid chromatographic method, using acetone as the internal standard

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements for oral solution packaged in single-unit containers
- **DELIVERABLE VOLUME (698):** Meets the requirements for oral solution packaged in multiple-unit containers

SPECIFIC TESTS

- **pH (791):** 1.4–4.6

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**
USP Oxycodone RS

Oxycodone Hydrochloride Tablets

DEFINITION

Oxycodone Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the oxycodone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Mobile phase: Acetonitrile, 0.01 M sodium 1-heptanesulfonate, and glacial acetic acid (25:74:1). Adjust this mixture with 5 N sodium hydroxide to a pH of 3.5.

Standard solution: 0.045 mg/mL of USP Oxycodone RS in *Mobile phase*

Sample solution: 0.05 mg/mL of oxycodone hydrochloride in *Mobile phase* from NLT 20 finely powdered Tablets. Initially add 50% of the volume of the flask of *Mobile phase*, sonicate for about 5 min, and shake by mechanical means for about 15 min. Dilute with *Mobile phase* to volume, and pass a portion of this mixture through a filter of 0.5- μ m or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1.7 mL/min

Injection size: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for the oxycodone peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxycodone hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of oxycodone hydrochloride, 351.82

M_{r2} = molecular weight of the oxycodone base, 315.37

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: Water; 500 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: Prepare a known concentration of USP Oxycodone RS in 0.1 N hydrochloric acid.

Sample solution: Sample per *Dissolution* (711). Dilute with *Medium* as needed.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: Maximum at about 225 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 70% (Q) of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**
USP Oxycodone RS

Oxycodone Hydrochloride Extended-Release Tablets

DEFINITION

Oxycodone Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY• **PROCEDURE**

Buffer solution: 2 g/L of sodium heptanesulfonate in water. Add 13.3 mL/L of glacial acetic acid, and adjust with 5 N sodium hydroxide solution to a pH of 3.50 ± 0.05 .

Mobile phase: Acetonitrile and *Buffer solution* (1:3)

Standard solution: 0.036 mg/mL of USP Oxycodone RS in *Mobile phase*

Sample stock solution: Transfer 10 Tablets into an appropriate volumetric flask, and add a volume of a mixture of methanol and acetonitrile (1:1) equivalent to 50% of the volumetric flask volume. Sonicate for 10 min, and stir for 20 min. Dilute with *Buffer solution* to volume.

Sample solution: 0.04 mg/mL of oxycodone hydrochloride from the *Sample stock solution*, diluted with *Mobile phase* to volume. Pass through a suitable filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm \times 15-cm; packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 4000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

M_{r1} = molecular weight of oxycodone hydrochloride, 351.82

M_{r2} = molecular weight of oxycodone base, 315.37

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)****Test 1**

Medium: Simulated gastric fluid (without enzymes); 900 mL

Apparatus 1: 100 rpm

Times: 1, 2, 4, 6, and 8 h for Tablets labeled to contain 10, 20, or 40 mg; 1, 2, 4, and 6 h for Tablets labeled to contain 80 mg

Standard stock solution

Tablets labeled to contain 10 mg: 398 μ g/mL of USP Oxycodone RS in *Medium*

Tablets labeled to contain 20, 40, or 80 mg: 796 μ g/mL of USP Oxycodone RS in *Medium*

Standard solution: Dilute the appropriate *Standard stock solution* with *Medium* to obtain solutions containing (L/900) mg/mL, with L as the label claim in mg/ Tablet.

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: About 226 nm (shoulder)

Cell

For Tablets labeled to contain 10, 20, or 40 mg: 1.0 cm

For Tablets labeled to contain 80 mg: 0.5 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (A_U/A_S) \times C_S \times D \times (M_{r1}/M_{r2})$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

D = dilution factor of the *Sample solution*

M_{r1} = molecular weight of oxycodone hydrochloride, 351.82

M_{r2} = molecular weight of oxycodone base, 315.37

For Tablets labeled to contain 10, 20, or 40 mg, calculate the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) released at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_3)] + (C_i \times V_3)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_3)]] + [(C_2 + C_i) \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times [V - (3 \times V_3)]] + [(C_3 + C_2 + C_i) \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_5 = \{[C_5 \times [V - (4 \times V_3)]] + [(C_4 + C_3 + C_2 + C_i) \times V_3]\} \times (1/L) \times 100$$

C_i = concentration of oxycodone hydrochloride in the portion of sample withdrawn at time point i (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_3 = volume of the *Sample solution* withdrawn from the *Medium* (mL)

For Tablets labeled to contain 80 mg, calculate the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) released at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_3)] + (C_i \times V_3)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_3)]] + [(C_2 + C_i) \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times [V - (3 \times V_3)]] + [(C_3 + C_2 + C_i) \times V_3]\} \times (1/L) \times 100$$

C_i = concentration of oxycodone hydrochloride in the portion of sample withdrawn at time point i (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_s = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 1* for Tablets labeled to contain 10, 20, or 40 mg; see *Table 2* for Tablets labeled to contain 80 mg.

Table 1

Time Point (i)	Time (h)	Amount Released (%)
1	1	20–40
2	2	35–55
3	4	55–75
4	6	70–90
5	8	NLT 80

Table 2

Time Point (i)	Time (h)	Amount Released (%)
1	1	25–45
2	2	45–65
3	4	65–85
4	6	NLT 80

The percentages of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$), released at the times specified, conform to *Acceptance Table 2* in *Dissolution* (711).

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution* Test 2.

Medium: Simulated gastric fluid (without enzymes); 900 mL

Apparatus 1: 100 rpm

Times: 1, 4, and 12 h

0.85% Phosphoric acid: 10 mL/L of phosphoric acid in water

Mobile phase: Weigh 23.1 g of monobasic potassium phosphate into a 4-L flask, and dissolve with 3400 mL of water. Add 4 mL of triethylamine, and adjust with 0.85% Phosphoric acid to a pH of 3.0 ± 0.1 . Add 600 mL of methanol and 20 mL of *tert*-butyl methyl ether, and mix well.

Standard stock solution: 0.9 mg/mL of USP Oxycodone RS in 0.85% Phosphoric acid

Standard solution: Dilute the *Standard stock solution*, quantitatively and stepwise, with *Medium* to obtain a solution having a concentration of 40% of the Tablet label claim. [NOTE—This solution is stable for two weeks at room temperature.]

Sample solution: Pass the solution under test through a suitable filter of 0.45- μ m pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L1

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 50 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor: NLT 0.5

Tailing factor: 0.75–1.5

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

M_{r1} = molecular weight of oxycodone hydrochloride, 351.82

M_{r2} = molecular weight of oxycodone base, 315.37

Calculate the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) released at each time point (i):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_s)] + (C_i \times V_s)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_s)]] + [(C_2 + C_i) \times V_s]\} \times (1/L) \times 100$$

C_i = concentration of oxycodone hydrochloride in the portion of sample withdrawn at time point i (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

V_s = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See *Table 3* for Tablets labeled to contain 10 mg; see *Table 4* for Tablets labeled to contain 20 mg; see *Table 5* for Tablets labeled to contain 40 mg; see *Table 6* for Tablets labeled to contain 80 mg.

Table 3

Time Point (i)	Time (h)	Amount Released (%)
1	1	29–49
2	4	58–78
3	12	NLT 85

Table 4

Time Point (i)	Time (h)	Amount Released (%)
1	1	33–53
2	4	63–83
3	12	NLT 85

Table 5

Time Point (<i>t</i>)	Time (h)	Amount Released (%)
1	1	37–57
2	4	68–88
3	12	NLT 85

Table 6

Time Point (<i>t</i>)	Time (h)	Amount Released (%)
1	1	31–51
2	4	61–81
3	12	NLT 85

The percentages of the labeled amount of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$), released at the times specified, conform to Acceptance Table 2 in Dissolution (711).

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

- **LIMIT OF OXYCODONE RELATED COMPOUND B (OXYCODONE N-OXIDE)**

Diluent: 10 mL/L of phosphoric acid in water

Buffer: 6.8 g/L of monobasic potassium phosphate.

Add 1.2 mL of triethylamine, and adjust with *Diluent* to a pH of 3.0 ± 0.1 .

Mobile phase: Methanol, *tert*-butyl methyl ether, and *Buffer* (30:1:170)

Standard solution: 0.18 mg/mL of USP Oxycodone RS and 0.002 mg/mL of USP Oxycodone Related Compound B RS in *Diluent*. [NOTE—Prepare fresh daily.]

Sample stock solution: Transfer 10 Tablets into a 500-mL volumetric flask, add 50 mL of *Diluent* and 50 mL of alcohol, and sonicate for 90 min to extract the active ingredient. Dilute with *Diluent* to volume.

Sample solution: 0.2 mg/mL of oxycodone hydrochloride from the *Sample stock solution* in *Diluent*. Pass a portion of the solution through a suitable filter, and use the filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 230 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L1

Column temperature: 60°

Flow rate: 1.0 mL/min

Injection volume: 50 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 4.5 between the oxycodone and oxycodone related compound B peaks

Relative standard deviation: NMT 3.0% for oxycodone related compound B

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxycodone related compound B in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of oxycodone related compound B from the *Sample solution*

r_S = peak area of oxycodone related compound B from the *Standard solution*

C_S = concentration of USP Oxycodone Related Compound B RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxycodone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution Test* is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
USP Oxycodone RS
USP Oxycodone Related Compound B RS
4,5a-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one *N*-oxide.
 $C_{18}H_{21}NO_5$ 331.36

Oxycodone and Acetaminophen Capsules

DEFINITION

Oxycodone and Acetaminophen Capsules contain Oxycodone Hydrochloride and Acetaminophen, or Oxycodone Hydrochloride, Oxycodone Terephthalate, and Acetaminophen. Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of oxycodone hydrochloride or oxycodone hydrochloride and oxycodone terephthalate, calculated as total oxycodone ($C_{18}H_{21}NO_4$), and NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution A: 0.5 mg/mL of USP Oxycodone RS in a mixture of methanol and water (4:1)

Standard solution B: 0.5/ mg/mL of USP Acetaminophen RS in a mixture of methanol and water (4:1).

[NOTE— r is the ratio of the labeled amount, in mg, of acetaminophen to the labeled amount, in mg, of oxycodone per Capsule.]

Sample solution: Nominally equivalent to 0.5 mg/mL of oxycodone from Capsules in a mixture of methanol and water (4:1). Sonicate for 5 min, and shake by mechanical means for 15 min. Allow to settle, and use the clear supernatant.

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Mode: TLC

Adsorbent: 0.25-mm layer of silica gel mixture

Application volume: 20 μ L

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (4:1:2)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Proceed as directed in the chapter. Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Mark the solvent front, and allow the plate to air-dry for about 30 min. Expose the plate to iodine vapors in a closed chamber, and locate the spots.

Acceptance criteria: The R_f values of the principal spots from the *Sample solution* correspond to those from the respective *Standard solutions*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Solution A: Methanol and 0.05 M dibasic potassium phosphate (1:9). Adjust with phosphoric acid to a pH of 4.0.

Buffer: 0.95 mg/mL of monobasic potassium phosphate in water, phosphoric acid, and *n*-nonylamine (1000:1:1). Prepare as follows: Add 950 mg of monobasic potassium phosphate to 1000 mL of water. Add 1 mL of phosphoric acid, and stir until dissolved. While stirring, add 1 mL of *n*-nonylamine, and stir until a clear solution is obtained. Adjust with potassium hydroxide TS to a pH of 4.9 ± 0.1 .

Mobile phase: Methanol and Buffer (1:9)

Oxycodone standard stock solution: 0.075 mg/mL of USP Oxycodone RS in Solution A

Standard stock solution: 0.03/ mg/mL of USP Acetaminophen RS and 0.03 mg/mL of USP Oxycodone RS in Solution A. Prepare by adding 40% of the flask volume of Solution A to the appropriate quantity of USP Acetaminophen RS, and then adding 40% of the flask volume of Oxycodone standard stock solution and diluting with Solution A to volume. [NOTE—*J* is the ratio of the labeled amount of acetaminophen, in mg, to that of oxycodone equivalent.]

Standard solution: 0.003/ mg/mL of USP Acetaminophen RS and 0.003 mg/mL of USP Oxycodone RS in Mobile phase from Standard stock solution

Sample stock solution: Nominal equivalent of 0.03 mg/mL of oxycodone, from Capsules (NLT 20), in Solution A in a suitable container. Shake by mechanical means for 1 h.

Sample solution: 0.003 mg/mL of oxycodone in Mobile phase from Sample stock solution. Pass the resulting solution through a membrane filter of 0.5- μ m or finer pore size, discarding the first 10 mL of the filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 40°

Flow rate: 2 mL/min

Injection size: 20 μ L

System suitability

Sample: Standard solution

[NOTE—The relative retention times for oxycodone and acetaminophen are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.4 between acetaminophen and oxycodone

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of oxycodone ($C_{18}H_{21}NO_4$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxycodone from the Sample solution

r_S = peak response of oxycodone from the Standard solution

C_S = concentration of USP Oxycodone RS in the Standard solution (mg/mL)

C_U = nominal concentration of oxycodone in the Sample solution (mg/mL)

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the Sample solution

r_S = peak response of acetaminophen from the Standard solution

C_S = concentration of USP Acetaminophen RS in the Standard solution (mg/mL)

C_U = nominal concentration of acetaminophen in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amounts of oxycodone hydrochloride or oxycodone hydrochloride and oxycodone terephthalate, calculated as total oxycodone ($C_{18}H_{21}NO_4$), and 90.0%–110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$)

PERFORMANCE TESTS**• DISSOLUTION, Procedure for a Pooled Sample (711)**

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Sample solution: Sample per Dissolution (711). Dilute with Medium as needed.

Analysis: Determine the amounts of oxycodone ($C_{18}H_{21}NO_4$) and acetaminophen ($C_8H_9NO_2$) dissolved, using the procedure in the Assay, making any necessary volumetric adjustments, including adjusting the solution under test to a pH of about 5.5 before injecting.

Tolerances: NLT 75% (Q) of the labeled amounts of oxycodone ($C_{18}H_{21}NO_4$) and acetaminophen ($C_8H_9NO_2$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.**• USP REFERENCE STANDARDS (11)**

USP Acetaminophen RS

USP Oxycodone RS

Oxycodone and Acetaminophen Tablets**DEFINITION**

Oxycodone and Acetaminophen Tablets contain Oxycodone Hydrochloride and Acetaminophen. Tablets contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of oxycodone ($C_{18}H_{21}NO_4$), and NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION**• A. THIN-LAYER CHROMATOGRAPHY**

Standard solution A: 0.5 mg/mL of USP Oxycodone RS in a mixture of methanol and water (4:1)

Standard solution B: 0.5/ mg/mL of USP Acetaminophen RS in a mixture of methanol and water (4:1).

[NOTE—*J* is the ratio of the labeled amount, in mg, of acetaminophen to the labeled amount, in mg, of oxycodone per Tablet.]

Sample solution: Nominally equivalent to 2.5 mg of oxycodone from powdered Tablets in a 5-mL mixture of methanol and water (4:1). Sonicate for 5 min, and shake by mechanical means for 15 min. Allow to settle, and use the clear supernatant.

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Adsorbent: 0.25-mm layer of silica gel mixture

Application volume: 20 μ L

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (4:1:2)

Analysis

Samples: *Standard solution A, Standard solution B, and Sample solution*

Proceed as directed in the chapter. Develop the chromatographic plate until the solvent front has moved about three-fourths of the length of the plate. Mark the solvent front, and allow the plate to air-dry for about 30 min. Expose the plate to iodine vapors in a closed chamber, and locate the spots.

Acceptance criteria: The R_f values of the principal spots from the *Sample solution* correspond to those from the respective *Standard solutions*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY• **PROCEDURE**

Solution A: Methanol and 0.05 M dibasic potassium phosphate (1:9). Adjust with phosphoric acid to a pH of 4.0.

Buffer: 0.95 mg/mL of monobasic potassium phosphate in water, phosphoric acid, and *n*-nonylamine (1000:1:1). Prepare as follows: Add 950 mg of monobasic potassium phosphate to 1000 mL of water. Add 1 mL of phosphoric acid, and stir until dissolved. While stirring, add 1 mL of *n*-nonylamine, and stir until a clear solution is obtained. Adjust with potassium hydroxide TS to a pH of 4.9 ± 0.1 .

Mobile phase: Methanol and *Buffer* (1:9)

Oxycodone standard stock solution: 0.075 mg/mL of USP Oxycodone RS in *Solution A*

Standard stock solution: 0.03 mg/mL of USP Acetaminophen RS and 0.03 mg/mL of USP Oxycodone RS in *Solution A*. Prepare by adding 40% of the flask volume of *Solution A* to the appropriate quantity of USP Acetaminophen RS, and then adding 40% of the flask volume of *Oxycodone standard stock solution* and diluting with *Solution A* to volume. [NOTE—] is the ratio of the labeled amount, in mg, of acetaminophen to that of oxycodone equivalent.]

Standard solution: 0.003 mg/mL of USP Acetaminophen RS and 0.003 mg/mL of USP Oxycodone RS in *Mobile phase* from *Standard stock solution*

Sample stock solution: Nominal equivalent of 0.03 mg/mL of oxycodone, from powdered Tablets (NLT 20), in *Solution A* in a suitable container. Shake by mechanical means for 1 h.

Sample solution: 0.003 mg/mL of oxycodone in *Mobile phase* from *Sample stock solution*. Pass the resulting solution through a membrane filter of 0.5- μ m or finer pore size, discarding the first 10 mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 40°

Flow rate: 2 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for oxycodone and acetaminophen are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.4 between acetaminophen and oxycodone

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxycodone ($C_{18}H_{21}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxycodone from the *Sample solution*

r_S = peak response of oxycodone from the *Standard solution*

C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxycodone in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*

r_S = peak response of acetaminophen from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of oxycodone ($C_{18}H_{21}NO_4$), and 90.0%–110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$)

PERFORMANCE TESTS• **DISSOLUTION, Procedure for a Pooled Sample (711)**

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Sample solution: Sample per *Dissolution* (711). Dilute with *Medium* as needed.

Analysis: Determine the amounts of oxycodone ($C_{18}H_{21}NO_4$) and acetaminophen ($C_8H_9NO_2$) dissolved, using the procedure in the Assay, and making any necessary volumetric adjustments, including adjusting the solution under test to a pH of about 5.5 before injecting.

Tolerances: NLT 75% (Q) of the labeled amounts of oxycodone ($C_{18}H_{21}NO_4$) and acetaminophen ($C_8H_9NO_2$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.• **LABELING:** The Tablets may be labeled to indicate the content of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$) equivalent. Each mg of oxycodone is equivalent to 1.116 mg of oxycodone hydrochloride.• **USP REFERENCE STANDARDS (11)**

USP Acetaminophen RS

USP Oxycodone RS

Oxycodone and Aspirin Tablets

» Oxycodone and Aspirin Tablets contain Oxycodone Hydrochloride and Aspirin, or Oxycodone Hydrochloride, Oxycodone Terephthalate, and Aspirin. Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of oxycodone ($C_{18}H_{21}NO_4$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ($C_9H_8O_4$).

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—Label the Tablets to state both the content of the oxycodone active moiety and the content or contents of the salt or salts of oxycodone used in formulating the article.

USP Reference standards (11)—

USP Aspirin RS
USP Oxycodone RS
USP Salicylic Acid RS

Identification—The retention times of the oxycodone peak and the aspirin peak in the chromatograms of the respective *Assay preparations* correspond to those of the corresponding analytes of the respective *Standard preparations*, as obtained in the *Assay for oxycodone* and the *Assay for aspirin*, respectively.

Dissolution, *Procedure for a Pooled Sample* (711)—

Medium: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of 4.50 ± 0.05 ; 500 mL.

Apparatus 1: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{18}H_{21}NO_4$ dissolved using the method for *Assay for oxycodone*, making any necessary volumetric adjustments. Determine the amount of $C_9H_8O_4$ dissolved from UV absorbances at the wavelength of the isobestic point of aspirin and salicylic acid at about 265 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Aspirin RS in the same medium. [NOTE—Prepare the *Standard solution* at the time of use. An amount of alcohol not to exceed 1% of the total volume of the *Standard solution* may be used to bring the Reference Standard into solution prior to dilution with *Medium*.]

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{18}H_{21}NO_4$ is dissolved in 30 minutes and not less than 75% (Q) of the labeled amount of $C_9H_8O_4$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Salicylic acid—

Mobile phase—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Diluting solution—Prepare a mixture of acetonitrile and formic acid (99:1).

Standard preparation—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Diluting solution* to obtain a solution having a known concentration of about 0.008 mg per mL.

Test preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 380 mg of aspirin, to a 100-mL volumetric flask, add about 20 mL of *Diluting solution*, and sonicate for about 15 minutes. Dilute with *Diluting solution* to volume, and mix. Centrifuge a portion of this mixture, and use the clear supernatant as the *Test preparation*.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 299-nm detector and a 3.9-mm \times 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 4.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Test preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the salicylic acid peaks. Calculate the per-

centage of salicylic acid in the portion of Tablets taken by the formula:

$$10,000(C/a)(r_u/r_s)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*, a is the quantity, in mg, of aspirin in the portion of Tablets taken, as determined in the *Assay for aspirin*, and r_u and r_s are the salicylic acid peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 3.0% is found.

Assay for aspirin—[NOTE—Volumetric flasks should be dried at 105° for not less than 1 hour, and cooled in a desiccator before use.]

Mobile phase—Prepare a mixture of n-heptane and glacial acetic acid (96:4), and filter through a filter of 0.5 μ m or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard solution—Prepare solution of 1-naphthol in chloroform containing about 1 mg per mL. [NOTE—Protect this solution from light.]

Standard preparation—Transfer about 163 mg of USP Aspirin RS, accurately weighed, to a 50-mL volumetric flask. Add 2.5 mL of glacial acetic acid, and swirl. Add 25 mL of chloroform, and shake for 10 minutes. Add 5.0 mL of *Internal standard solution*, dilute with chloroform to volume, and mix. [NOTE—Protect this solution from light.]

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a 100-mL volumetric flask, add 5 mL of glacial acetic acid, and swirl. Add 50 mL of chloroform, and shake for 10 minutes. Add 10.0 mL of the *Internal standard solution*, dilute with chloroform to volume, mix, and filter. [NOTE—Prepare the *Assay preparation* and the *Standard preparation* concomitantly, and protect from light.]

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 300-nm detector and a 4.6-mm \times 25-cm column containing packing L3. The flow rate is about 4 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R, between the 1-naphthol peak and the aspirin peak is not less than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.65 for 1-naphthol and 1.0 for aspirin. Calculate the quantity, in mg, of Aspirin ($C_9H_8O_4$) in the portion of Tablets taken by the formula:

$$100C(R_u/R_s)$$

in which C is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*, and R_u and R_s are the ratios of the aspirin peak response to the 1-naphthol peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for oxycodone—

Mobile phase—Dissolve 2.2 g of sodium 1-octanesulfonate in 740 mL of water, add 260 mL of methanol, 10 mL of glacial acetic acid, and 0.1 mL of triethylamine. Mix, and adjust with 5 N sodium hydroxide to a pH of 6.5 ± 0.1 . Filter through a suitable filter of 0.5 μ m or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Diluting solution—Use 0.1 N hydrochloric acid.

Internal standard solution—Transfer about 50 mg of ethylparaben to a 500-mL volumetric flask, add 10 mL of methanol, and swirl to dissolve. Dilute with *Diluting solution* to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Oxycodone RS in *Diluting solution*, and dilute quantitatively with *Diluting solution* to obtain a stock solution having a known concentration of about 0.75 mg per mL. Transfer 15.0 mL of this stock solution to a second 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Diluting solution* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.112 mg of USP Oxycodone RS per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 11.2 mg of oxycodone, to a suitable glass-stoppered conical flask, add 50.0 mL of *Diluting solution*, and shake by mechanical means for about 30 minutes. Filter this solution, transfer 25.0 mL of the clear filtrate to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with *Diluting solution* to volume, and mix. Use this solution as the *Assay preparation*.

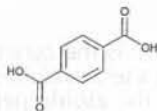
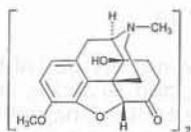
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 15-cm column that contains packing L1 and is maintained at a temperature of 50 ± 1.0°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the column efficiency, determined from the oxycodone peak, is not less than 1800 theoretical plates, the resolution, *R*, between the oxycodone and the ethylparaben peaks is not less than 6, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 30 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for oxycodone and 1.0 for ethylparaben. Calculate the quantity, in mg, of oxycodone (C₁₈H₂₁NO₄) in the portion of Tablets taken by the formula:

$$100C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Oxycodone RS in the *Standard preparation*, and *R_U* and *R_S* are the ratios of the responses of the oxycodone peak and the ethylparaben peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Oxycodone Terephthalate



(C₁₈H₂₁NO₄)₂ · C₈H₆O₄ 796.86
Morphinan-6-one, 4,5-epoxy-14-hydroxy-3-methoxy-17-methyl-, 1,4-benzenedicarboxylate (2:1 salt), (5α); 4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one 1,4-benzenedicarboxylate (2:1 salt) [64336-55-6].

DEFINITION

Oxycodone Terephthalate contains NLT 97.0% and NMT 103.0% of oxycodone terephthalate (C₁₈H₂₁NO₄)₂ · C₈H₆O₄, calculated on the dried basis.

IDENTIFICATION

A. MELTING RANGE OR TEMPERATURE (741)

Sample solution: Transfer 50 mL of the filtrate retained from the test for *Content of Terephthalate Acid* to a 125-mL conical flask. Render the solution alkaline with 6 N ammonium hydroxide. Allow the mixture to stand until a precipitate is formed. Filter, wash the precipitate with 50 mL of cold water, and dry for 2 h at 105°.

Acceptance criteria: The precipitate melts between 218° and 223°, but the range between the beginning and end of the melting does not exceed 2°.

B. INFRARED ABSORPTION (197K)

Sample: Use a portion of the dried precipitate obtained in *Identification test A*.

Acceptance criteria: Meets the requirements

C. ULTRAVIOLET ABSORPTION (197U)

Sample solution: 150 µg/mL in 0.1 N hydrochloric acid

Acceptance criteria: Exhibits a maxima at 280 nm

ASSAY

PROCEDURE

Mobile phase: To 2.2 g of sodium 1-octanesulfonate in 740 mL of water add 260 mL of methanol, 10 mL of glacial acetic acid, and 0.1 mL of triethylamine. Mix, and adjust with 5 N sodium hydroxide to a pH of 6.5 ± 0.1. Pass through a filter of 0.5-µm or finer pore size.

Diluent: 0.1 N hydrochloric acid

Internal standard solution: 0.1 mg/mL of ethylparaben prepared by dissolving in 2% of the flask volume of methanol and diluting with *Diluent* to volume

Standard stock solution: 0.75 mg/mL of USP Oxycodone RS in *Diluent*

Standard solution: 0.11 mg/mL of USP Oxycodone RS prepared as follows. Transfer 15.0 mL of *Standard stock solution* to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.

Sample stock solution: 0.71 mg/mL of Oxycodone Terephthalate in *Diluent*. Filter, discarding the first 5 mL.

Sample solution: 0.14 mg/mL of Oxycodone Terephthalate prepared as follows. Transfer 10.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 15-cm; packing L1

Column temperature: 50 ± 1.0°

Flow rate: 1 mL/min

Run time: Twice the retention time of the main oxycodone peak

Injection size: 30 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 6 between oxycodone and ethylparaben

Column efficiency: NLT 1800 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxycodone terephthalate (C₁₈H₂₁NO₄)₂ · C₈H₆O₄ in the portion of Oxycodone Terephthalate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of oxycodone to ethylparaben from the *Sample solution*

R_S = peak response ratio of oxycodone to ethylparaben from the *Standard solution*

C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)

- C_U = concentration of oxycodone in the *Sample solution* (mg/mL)
 M_{r1} = one-half of the molecular weight of oxycodone terephthalate, 398.43
 M_{r2} = molecular weight of oxycodone, 315.37
Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 1%

- **ORGANIC IMPURITIES**

Solution A: 2.2 g of sodium 1-octanesulfonate in 850 mL of water. Add 150 mL of methanol, 20 mL of glacial acetic acid, and 1.0 mL of triethylamine. Pass through a filter of 0.5- μ m or finer pore size.

Solution B: 2.2 g of sodium 1-octanesulfonate in 500 mL of water. Add 500 mL of methanol, 20 mL of glacial acetic acid, and 1.0 mL of triethylamine. Pass through a filter of 0.5- μ m or finer pore size.

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
30	80	20
50	0	100
55	0	100

Diluent: 0.1 N hydrochloric acid

Standard stock solution: 0.9 mg/mL of USP Oxycodone RS in *Diluent*

Standard solution: 0.09 mg/mL of USP Oxycodone RS from the *Standard stock solution*, prepared by adding to 20% of the flask volume of methanol, and diluting with *Diluent* to volume

System suitability stock solution: 0.05 mg/mL of 4-hydroxybenzoic acid isopropyl ester in methanol

System suitability solution: 0.01 mg/mL of 4-hydroxybenzoic acid isopropyl ester and 0.09 mg/mL of USP Oxycodone RS in *Diluent* from the *System suitability stock solution* and *Standard stock solution*, respectively

Sample solution: 11 mg/mL of Oxycodone Terephthalate in methanol prepared as follows. Transfer the required amount of sample to a suitable volumetric flask. Add 80% of the flask volume of methanol, and shake by mechanical means for about 20 min to dissolve. Dilute with methanol to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm \times 15-cm; packing L1

Column temperature: 45 \pm 1°

Flow rate: 1.5 mL/min

Injection size: 25 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 8 between the oxycodone and 4-hydroxybenzoic acid isopropyl ester peaks, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of an individual impurity from the *Sample solution*

r_S = peak area of oxycodone from the *Standard solution*

C_S = concentration of USP Oxycodone RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxycodone Terephthalate in the *Sample solution* (mg/mL)

M_{r1} = one-half of the molecular weight of oxycodone terephthalate, 398.43

M_{r2} = molecular weight of oxycodone, 315.37

[NOTE—If any impurity is found having a retention time of about 2 in relation to that of the oxycodone peak, divide its apparent percentage by 4.8.]

Acceptance criteria

Individual impurities: NMT 1.0%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **CONTENT OF TEREPHTHALIC ACID**

Sample solution: Transfer 1 g into a 50-mL beaker. Add 25 mL of 0.2 N hydrochloric acid, and heat to boiling with continuous stirring. Cover the beaker with a watch glass, and allow to cool to room temperature. Pass the suspension through a tared, medium-porosity filtering crucible. Transfer any material remaining in the beaker to the crucible with the aid of small portions of cold 0.2 N hydrochloric acid. Wash the material in the crucible with several portions of cold 0.2 N hydrochloric acid. [NOTE—Reserve the combined filtrates for use in *Identification test A*.]

Analysis: Dry the material in the crucible at 105° for 1 h, allow to cool, and reweigh. The material in the crucible is terephthalic acid. Determine the weight of terephthalic acid, and calculate the percentage of terephthalic acid.

Acceptance criteria: Between 20.2% and 21.5% of terephthalic acid ($C_8H_6O_4$) in Oxycodone Terephthalate on the dried basis

- **LOSS ON DRYING** (731): Dry a sample at 105° for 4 h: it loses NMT 1.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS** (11)

USP Oxycodone RS

Oxygen

O₂

32.00

Oxygen [7782-44-7].

DEFINITION

Oxygen contains NLT 99.0% of oxygen (O₂) by volume.

IDENTIFICATION

- **A.** The paramagnetic signal exhibited by the *Sample gas* in the *Assay* confirms the presence of oxygen.
- **B.** The *Sample gas* in the *Assay* meets the *Acceptance criteria*.

ASSAY

- **PROCEDURE**

The certified standards called for in the following test are listed in *Reagents, Indicators, and Solutions*.

Zero gas: Nitrogen certified standard

Span gas: Oxygen certified standard

Sample gas: Oxygen under test

Mode: Paramagnetic oxygen measurement (see *Medical Gases Assay* (415))

Analysis: Determine the concentration of oxygen in percentage by volume in Oxygen using a suitable paramagnetic analyzer.

Acceptance criteria: NLT 99.0% by volume

IMPURITIES

See *Impurities Testing in Medical Gases Assay* (413). The detector tubes called for in the following tests are listed in *Reagents, Indicators, and Solutions*. If the label indicates that Oxygen is produced by the air-liquefaction process, then the *Impurities* tests are not required.

- **LIMIT OF CARBON DIOXIDE**

Sample: Detector tube manufacturer's recommended volume, $\pm 5\%$ of Oxygen

Analysis: Pass the *Sample* through a carbon dioxide detector tube at the rate specified for the tube by the detector tube manufacturer.

Acceptance criteria: NMT 300 ppm

- **LIMIT OF CARBON MONOXIDE**

Sample: Detector tube manufacturer's recommended volume, $\pm 5\%$ of Oxygen

Analysis: Pass the *Sample* through a carbon monoxide detector tube at the rate specified for the tube by the detector tube manufacturer.

Acceptance criteria: NMT 10 ppm

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in pressurized containers. Container connections shall be appropriate for Oxygen. Adaptors shall not be used to connect containers to patient-use supply system piping or equipment.
- **LABELING:** Label states if Oxygen was produced by the air-liquefaction process. Where it is piped directly from the cylinder or storage tank to the patient point of use, label each outlet "Oxygen".

Oxygen 93 Percent

DEFINITION

Oxygen 93 Percent is Oxygen produced from air by the molecular sieve process. It contains NLT 90.0% and NMT 96.0% by volume of oxygen (O_2), the remainder consisting mostly of argon and nitrogen.

IDENTIFICATION

- **A.** The paramagnetic signal exhibited by the *Sample gas* in the Assay confirms the presence of oxygen.
- **B.** The *Sample gas* in the Assay meets the *Acceptance criteria*.

ASSAY

- **PROCEDURE**

The certified standards called for in the following test are listed in *Reagents, Indicators, and Solutions*.

Zero gas: Nitrogen certified standard

Span gas: 93% Oxygen certified standard

Sample gas: Oxygen 93 Percent under test

Mode: Paramagnetic oxygen measurement (see *Medical Gases Assay* (415))

Analysis: Determine the concentration of oxygen in percentage by volume in Oxygen 93 Percent using a suitable paramagnetic analyzer.

Acceptance criteria: 90.0%–96.0% oxygen by volume

IMPURITIES

See *Impurities Testing in Medical Gases Assay* (413). The detector tubes called for in the following tests are listed in *Reagents, Indicators, and Solutions*.

- **CARBON DIOXIDE**

Sample: Detector tube manufacturer's recommended volume $\pm 5\%$ of Oxygen 93 Percent

Analysis: Pass the *Sample* through a carbon dioxide detector tube at the rate specified for the tube by the detector tube manufacturer.

Acceptance criteria: NMT 300 ppm

- **CARBON MONOXIDE**

Sample: Detector tube manufacturer's recommended volume $\pm 5\%$ of Oxygen 93 Percent

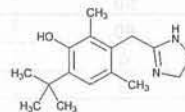
Analysis: Pass the *Sample* through a carbon monoxide detector tube at the rate specified for the tube by the detector tube manufacturer.

Acceptance criteria: NMT 10 ppm

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in pressurized containers. Container connections shall be appropriate for Oxygen 93 Percent. Adaptors shall not be used to connect containers to patient use supply system piping or equipment.
- **LABELING:** If Oxygen 93 Percent is piped from a remote location to the patient point of use, label each outlet "Oxygen 93 Percent".

Oxymetazoline Hydrochloride



$C_{16}H_{24}N_2O \cdot HCl$ 296.84

Phenol, 3-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-6-(1,1-dimethylethyl)-2,4-dimethyl-, monohydrochloride; 6-tert-Butyl-3-(2-imidazolin-2-ylmethyl)-2,4-dimethylphenol monohydrochloride [2315-02-8].

DEFINITION

Oxymetazoline Hydrochloride contains NLT 98.5% and NMT 101.5% of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride (191)**
Sample solution: 50 mg in 3 mL of water
Analysis: To the *Sample solution* add 1 mL of 6 N ammonium hydroxide. Filter, and acidify the filtrate with nitric acid.
Acceptance criteria: The filtrate meets the requirements.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, 1 M sodium acetate, glacial acetic acid, and water (40:10:4:46)

Standard solution: 0.5 mg/mL of USP Oxymetazoline Hydrochloride RS in *Mobile phase*

Sample solution: 0.5 mg/mL of Oxymetazoline Hydrochloride in *Mobile phase*

Chromatographic system
 (See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm \times 25-cm; packing L9

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.55% for five replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$) in the portion of Oxymetazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxymetazoline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxymetazoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1-Jan-2018)

- **ORGANIC IMPURITIES**

Buffer: 1.36 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: See Table 1. Return to the original conditions, and re-equilibrate the system.

Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	70	30
5	70	30
20	15	85
35	15	85

System suitability solution: 1.0 mg/mL of USP Oxymetazoline Hydrochloride RS and 1.5 µg/mL of USP Oxymetazoline Related Compound A RS in water

Standard solution: 1.0 µg/mL of USP Oxymetazoline Hydrochloride RS and 1.5 µg/mL of USP Oxymetazoline Related Compound A RS in water

Sample solution: 1.0 mg/mL of Oxymetazoline Hydrochloride in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between oxymetazoline related compound A and oxymetazoline peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of oxymetazoline related compound A in the portion of Oxymetazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of oxymetazoline related compound A from the *Sample solution*

r_S = peak response of oxymetazoline related compound A from the *Standard solution*

C_S = concentration of USP Oxymetazoline Related Compound A RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxymetazoline Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each individual unspecified impurity in the portion of Oxymetazoline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual unspecified impurity from the *Sample solution*

r_S = peak response of oxymetazoline from the *Standard solution*

C_S = concentration of USP Oxymetazoline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxymetazoline Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Oxymetazoline related compound A	0.9	0.15
Oxymetazoline	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

SPECIFIC TESTS

- **pH** (791)

Sample solution: 50 mg/mL in water

Acceptance criteria: 4.0–6.5

- **Loss on Drying** (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS** (11)

USP Oxymetazoline Hydrochloride RS

USP Oxymetazoline Related Compound A RS

N-(2-Aminoethyl)-2-[4-(*tert*-butyl)-3-hydroxy-2,6-dimethylphenyl]acetamide.

$C_{16}H_{26}N_2O_2$ 278.39

Oxymetazoline Hydrochloride Nasal Solution

DEFINITION

Oxymetazoline Hydrochloride Nasal Solution is a solution of Oxymetazoline Hydrochloride in water adjusted to a suitable tonicity. It contains NLT 90.0% and NMT 110.0% of the labeled amount of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, water, 1 M sodium acetate, and glacial acetic acid (40:46:10:4)

Standard solution: Prepare a solution of USP Oxymetazoline Hydrochloride RS in *Mobile phase*, having a

known concentration approximately equal to the labeled concentration of the Nasal Solution.

Sample solution: Nasal Solution

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; packing L9

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for five replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$) in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of oxymetazoline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH** (791): 4.0–6.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)
USP Oxymetazoline Hydrochloride RS

Oxymetazoline Hydrochloride Ophthalmic Solution

DEFINITION

Oxymetazoline Hydrochloride Ophthalmic Solution is a sterile, buffered solution of Oxymetazoline Hydrochloride in water adjusted to a suitable tonicity. It contains NLT 90.0% and NMT 110.0% of the labeled amount of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$). It contains a suitable preservative.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• **PROCEDURE**

Mobile phase: Methanol, 1 M sodium acetate, glacial acetic acid, and water (40:10:4:46)

Standard solution: Prepare a solution of USP Oxymetazoline Hydrochloride RS in *Mobile phase* having a known concentration approximately equal to the labeled concentration of the Ophthalmic Solution.

Sample solution: Use the Ophthalmic Solution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm or diode array. [NOTE—Use diode array detector to perform *Identification test B*.]

Column: 4.6-mm × 25-cm; packing L9

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for five replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Oxymetazoline Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxymetazoline hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

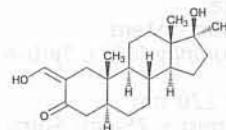
SPECIFIC TESTS

- **pH** (791): 5.8–6.8
- **STERILITY TESTS** (71): Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)
USP Oxymetazoline Hydrochloride RS

Oxymetholone



$C_{21}H_{32}O_3$

332.48

Androstan-3-one, 17-hydroxy-2-(hydroxymethylene)-17-methyl-, (5 α ,17 β)-;

17 β -Hydroxy-2-(hydroxymethylene)-17-methyl-5 α -androstan-3-one [434-07-1].

DEFINITION

Oxymetholone contains NLT 97.0% and NMT 103.0% of oxymetholone ($C_{21}H_{32}O_3$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

- **B. ULTRAVIOLET ABSORPTION** (197U)

Sample solution: 10 µg/mL in 0.01 N methanolic sodium hydroxide

Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Diluent: Alcohol and chloroform (50:50)

Standard solution: Prepare as directed in *Single-Steroid Assay* (511), using USP Oxymetholone RS.

Sample solution: Dissolve 20 mg of Oxymetholone, previously dried, in *Diluent*, and dilute with *Diluent* to 10.0 mL.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 315 nm

Cell: 1 cm

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in *Single-Steroid Assay* (511), *Procedure* through the third sentence of the second paragraph (ending with "50-mL centrifuge tube"), except use the *Standard solution* and the *Sample solution* in place of the *Standard Preparation* and the *Assay Preparation*, respectively. Use a mixture of benzene and alcohol (98:2) as the *Solvent*. To each tube add 25.0 mL of 0.01 N alcoholic sodium hydroxide, and shake for NLT 2 min. Centrifuge the tubes for 5 min, and determine the absorbances of the supernatants against the blank.

Calculate the percentage of oxymetholone ($C_{21}H_{32}O_3$) in the portion of Oxymetholone taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Oxymetholone RS in the *Standard solution* (mg/mL)

C_U = concentration of Oxymetholone in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** (741): 172°–180°

- **OPTICAL ROTATION, Specific Rotation** (781S)

Sample solution: 20 mg/mL in dioxane

Acceptance criteria: +34° to +38°

- **LOSS ON DRYING** (731)

Analysis: Dry under vacuum over phosphorus pentoxide for 4 h.

Acceptance criteria: NMT 1.0%

- **COMPLETENESS OF SOLUTION**

Sample solution: 20 mg/mL in dioxane

Acceptance criteria: The solution is clear and free from undissolved solid.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS** (11)

USP Oxymetholone RS

Oxymetholone Tablets

DEFINITION

Oxymetholone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxymetholone ($C_{21}H_{32}O_3$).

IDENTIFICATION

- **A. INFRARED ABSORPTION**

Sample: Nominally 50 mg of oxymetholone from powdered Tablets

Analysis: Mix the *Sample* with 15 mL of solvent hexane, and stir occasionally for 15 min. Centrifuge the mixture, and decant and discard the solvent hexane. Extract the residue with two 10-mL portions of solvent hexane, centrifuging and decanting as before, and discard the solvent hexane. Add 25 mL of chloroform to the residue, mix by shaking for 1–2 min, and filter. Evaporate

the filtrate to about 3 mL, add a few mL of solvent hexane to induce crystallization, and evaporate to dryness.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion prepared from the oxymetholone so obtained, and previously dried, exhibits maxima only at the same wavelengths as those of a similar preparation of USP Oxymetholone RS, crystallized from the same solvent mixture.

ASSAY

- **PROCEDURE**

Solution A: 4 g/L of sodium hydroxide in methanol

Solution B: 0.4 g/L of sodium hydroxide in methanol from *Solution A*

Standard solution: 10 µg/mL of USP Oxymetholone RS in *Solution B*. [NOTE—The solution is freshly prepared.]

Sample solution: Nominally 10 µg/mL of Oxymetholone prepared as follows. Transfer the equivalent of 20 mg of oxymetholone, from NLT 20 powdered Tablets, to a separator. Add 10 mL of water, and extract with three 25-mL portions of chloroform, filtering each extract through chloroform-washed cotton. Evaporate the combined chloroform extracts on a steam bath to dryness, reducing the application of heat as dryness is approached. Dissolve the residue in methanol, transfer to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of the solution to a 100-mL volumetric flask, add 10 mL of *Solution A*, and dilute with methanol to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 315 nm

Cell: 1 cm

Blank: *Solution B*

Analysis

Samples: *Standard solution* and *Sample solution*

Without delay, concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*.

Calculate the percentage of the labeled amount of oxymetholone ($C_{21}H_{32}O_3$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Oxymetholone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of oxymetholone in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** (711)

Medium: 0.05 M alkaline borate buffer, pH 8.5; 900 mL. [NOTE—See *Reagents, Indicators, and Solutions—Buffer Solutions*.]

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: USP Oxymetholone RS in *Medium*.

[NOTE—An amount of acetonitrile not to exceed 5% of the total volume of the *Standard solution* may be used to bring the Reference Standard into solution before dilution with the *Medium*.]

Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 313

Tolerances: NLT 75% (Q) of the labeled amount of oxymetholone ($C_{21}H_{32}O_3$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Solution A and Solution B: Prepare as directed in the Assay.

Standard solution: 10 µg/mL of USP Oxymetholone RS in *Solution B*. [NOTE—The solution is freshly prepared.]

Sample solution: Transfer 1 finely powdered Tablet to a 100-mL volumetric flask with the aid of about 75 mL of methanol. Heat the methanol to boiling, and allow to remain at a temperature just below the boiling point for 15 min with occasional swirling. Cool to room temperature, dilute with methanol to volume, and mix. Centrifuge a portion of the mixture until the solution becomes clear. Transfer a portion of the supernatant, nominally equivalent to 1 mg of oxymetholone, to a 100-mL volumetric flask. Add 10 mL of *Solution A*, and dilute with methanol to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 315 nm

Cell: 1 cm

Blank: *Solution B*

Analysis

Samples: *Standard solution* and *Sample solution*
Without delay, concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*.

Calculate the percentage of the labeled amount of oxymetholone (C₂₁H₃₂O₃) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Oxymetholone RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of oxymetholone in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

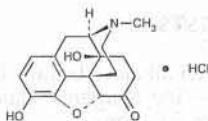
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**

USP Oxymetholone RS

Oxymorphone Hydrochloride



C₁₇H₁₉NO₄ · HCl 337.80
Morphinan-6-one, 4,5-epoxy-3,14-dihydroxy-17-methyl-, hydrochloride, (5α)-;

4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one hydrochloride [357-07-3].

DEFINITION

Oxymorphone Hydrochloride contains NLT 97.0% and NMT 102.0% of oxymorphone hydrochloride (C₁₇H₁₉NO₄ · HCl), calculated on the dried basis.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Chloride (191)**

Sample solution: Dissolve about 250 mg in 25 mL of water, and render the solution alkaline with a saturated

solution of sodium bicarbonate. Extract the liberated oxymorphone with two 15-mL portions of chloroform. Reserve the chloroform extracts for *Identification test B*. Acidify the aqueous phase with 2 N nitric acid.

Acceptance criteria: Meets the requirements

- **B.**

Standard solution: Similarly prepared as directed for the *Sample solution* by using USP Oxymorphone RS

Sample solution: Wash the combined chloroform extracts from *Identification test A* with 5 mL of water, and filter. Evaporate the chloroform solution on a steam bath nearly to dryness, then add a few mL of ether, and continue the evaporation with stirring until the solvent is removed. Dissolve the oxymorphone so obtained with alcohol-free chloroform to obtain a 1 in 50 solution.

Acceptance criteria: The IR absorption spectrum of the *Sample solution*, determined in a 0.5-mm cell, exhibits maxima only at the same wavelengths as those of the *Standard solution*.

- **C.**

Standard solution: Dissolve about 20 mg of USP Oxymorphone RS in 10 mL of 1 N hydrochloric acid, and dilute with water to 100.0 mL.

Sample solution: A solution (1 in 6500) in 0.1 N hydrochloric acid

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the *Standard solution*. The ratio A_{281}/A_{264} is 1.75 ± 0.2 .

- **D.**

Sample solution: Dissolve 10 mg in 1 mL of water.

Analysis: To the *Sample solution* add a few drops of ferric chloride TS.

Acceptance criteria: A blue color is produced immediately.

ASSAY

• PROCEDURE

Sample solution: Transfer about 700 mg of Oxymorphone Hydrochloride to a glass-stoppered flask containing 50 mL of glacial acetic acid and 10 mL of mercuric acetate TS. Add 3 mL of acetic anhydride and 1 drop of methyl violet TS.

Analysis: Titrate the *Sample solution* with 0.1 N perchloric acid VS to a clear blue color. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 33.78 mg of oxymorphone hydrochloride (C₁₇H₁₉NO₄ · HCl).

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.3%

- **ORDINARY IMPURITIES (466)**

Sample solution: Methanol

Standard solution: Methanol

Eluent: Dehydrated alcohol, cyclohexane, and ammonium hydroxide (10:5:1)

Visualization: 1

Acceptance criteria: Meets the requirements

- **LIMIT OF NONPHENOLIC SUBSTANCES**

Analysis: Dissolve 1 g in 15 mL of water. Add 5 mL of sodium hydroxide solution (2 in 25), and extract with three 10-mL portions of chloroform. Filter the combined extracts through a small chloroform-moistened filter paper, and wash the filtrate with 5 mL of water. Filter the chloroform layer through chloroform-moistened filter paper into a tared, 50-mL beaker, and evaporate on a steam bath with the aid of a gentle current of filtered air to dryness. Dry the beaker and residue at 105° for 1 h, and weigh.

Acceptance criteria: The residue so obtained does not exceed 15 mg.

SPECIFIC TESTS**• CONTENT OF CHLORIDE**

Sample solution: Dissolve about 300 mg in 50 mL of methanol in a glass-stoppered flask. Add 5 mL of glacial acetic acid and 3 drops of eosin Y TS.

Analysis: Titrate the *Sample solution* with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.

Acceptance criteria: 10.2%–10.8% on the dried basis

• OPTICAL ROTATION, Specific Rotation (781S)

Sample solution: 100 mg/mL in water

Acceptance criteria: -145° to -155°

• ACIDITY

Analysis: Dissolve 300 mg in 10 mL of water. Add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide.

Acceptance criteria: NMT 0.30 mL is required to produce a yellow color.

• LOSS ON DRYING (731)

Analysis: Dry at 105° for 18 h.

Acceptance criteria: NMT 8.0%

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25° , excursions permitted between 15° and 30° .

• USP REFERENCE STANDARDS (11)

USP Oxymorphone RS

Oxymorphone Hydrochloride Injection**DEFINITION**

Oxymorphone Hydrochloride Injection is a sterile solution of Oxymorphone Hydrochloride in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$).

IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- B.** The UV absorption spectra of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Protect all solutions containing oxymorphone from light and use clear glass HPLC vials.

Solution A: Dissolve 2.02 g of sodium 1-heptanesulfonate in 900 mL of water. Add 100 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.

Solution B: Dissolve 2.02 g of sodium 1-heptanesulfonate in 750 mL of water. Add 250 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
35	0	100
40	0	100
40.1	100	0
50.1	100	0

Diluent: Dissolve 2.02 g of anhydrous sodium 1-heptanesulfonate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.1.

Standard solution: 0.14 mg/mL of USP Oxymorphone RS prepared as follows. Transfer a suitable amount of USP Oxymorphone RS to a suitable volumetric flask. Add 50% of the flask volume of *Diluent*. Sonicate to dissolve, if necessary. Add 9% of the flask volume of acetonitrile. Cool to room temperature and dilute with *Diluent* to volume.

Sample solution: Nominally 0.15 mg/mL of oxymorphone hydrochloride from Injection prepared as follows. Transfer a suitable volume of the composite sample from NLT 20 ampules to a suitable volumetric flask. Dilute with *Solution A* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector

Assay: UV 230 nm

Identification test B: Diode array UV 200–360 nm

Column: 4.6-mm \times 7.5-cm; 3.5- μ m packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 30 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution*

r_S = peak response of oxymorphone from the *Standard solution*

C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxymorphone hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80

M_{r2} = molecular weight of oxymorphone, 301.34

Acceptance criteria: 93.0%–107.0%

IMPURITIES**• ORGANIC IMPURITIES**

Protect all solutions containing oxymorphone from light and use clear glass HPLC vials.

Solution A, Solution B, Mobile phase, Diluent, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability stock solution A: 0.2 mg/mL of USP Oxymorphone Related Compound A RS prepared as follows. Transfer a suitable amount of USP Oxymorphone Related Compound A RS to a suitable volumetric flask. Dissolve with 24% of the flask volume of 0.1 N hydrochloric acid and dilute with acetonitrile to volume.

System suitability stock solution B: 0.02 mg/mL of USP Oxymorphone Related Compound A RS in acetonitrile from *System suitability stock solution A*

System suitability stock solution C: 0.14 mg/mL of USP Oxymorphone RS prepared as follows. Transfer a suitable amount of USP Oxymorphone RS to a suitable volumetric flask. Add 50% of the flask volume of *Diluent*. Sonicate to dissolve if necessary. Add 9% of the flask volume of acetonitrile. Cool to room temperature and dilute with *Diluent* to volume.

System suitability solution: 0.0008 mg/mL of USP Oxymorphone Related Compound A RS in *System suitability stock solution C* from *System suitability stock solution B*

Standard solution: 0.00014 mg/mL of USP Oxymorphone RS prepared as follows. Dilute *System suitability stock solution C* with *Solution A*.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between oxymorphone related compound A and oxymorphone, *System suitability solution*

Relative standard deviation: NMT 10%, *Standard solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual degradation product in the portion of Injection taken:

$$\text{Result} = \left\{ \frac{r_U/F}{r_S + \Sigma(r_U/F)} \right\} \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*

F = relative response factor of each individual degradation product (see *Table 2*)

r_S = peak response of oxymorphone from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
10-Hydroxyoxymorphone ^a	0.59	1.0	0.20
Oxymorphone related compound A (oxymorphone N-oxide)	0.82	1.1	0.30
Oxymorphone	1.00	1.0	—
10-Ketooxymorphone ^b	1.37	0.83	0.30
Oxycodone ^c	1.97	1.0	—
1-Bromo-oxymorphone ^d	2.05	1.0	—
2,2'-Bisoxymorphone ^e	2.08	1.7	1.00
Any individual unspecified degradation product	—	1.0	0.50
Total degradation products	—	—	2.00

^a 4,5α-Epoxy-3,10,14-trihydroxy-17-methylmorphinan-6-one.

^b 4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6,10-dione.

^c Process impurities, not included in the total degradation products.

^d 1-Bromo-4,5α-epoxy-3,14-dihydroxy-17-methylmorphinan-6-one.

^e 2,2'-Bisoxymorphone.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 238.1 USP Endotoxin Units/mg of oxymorphone hydrochloride
- **pH (791):** 2.7–4.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products (1)*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers of Type I glass. Store at 25°,

excursions permitted between 15° and 30°, and protected from light.

• USP REFERENCE STANDARDS (11)

USP Endotoxin RS

USP Oxymorphone RS

USP Oxymorphone Related Compound A RS

4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one N-oxide.

C₁₇H₁₉NO₅ 317.34

Oxymorphone Hydrochloride Tablets

DEFINITION

Oxymorphone Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxymorphone hydrochloride (C₁₇H₁₉NO₄ · HCl).

IDENTIFICATION

- **A.** The retention time of the oxymorphone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the oxymorphone peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Protect all solutions containing oxymorphone from light and use clear glass HPLC vials.

Solution A: Dissolve 2.02 g of sodium 1-heptanesulfonate in 900 mL of water and add 100 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.

Solution B: Dissolve 2.02 g of sodium 1-heptanesulfonate in 750 mL of water and add 250 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
35	0	100
40	0	100
40.1	100	0
50.1	100	0

Standard solution: 0.14 mg/mL of USP Oxymorphone RS in *Solution A*. Sonicate to dissolve if necessary.

Sample solution: Nominally 0.16 mg/mL of oxymorphone hydrochloride in *Solution A* prepared as follows. Transfer NLT 8 Tablets to a suitable volumetric flask and add about 50% of the final volume of *Solution A*. Sonicate for at least 15 min with occasional vigorous shaking until the Tablets disintegrate completely. Then shake for at least 20 min. Immediately dilute with *Solution A* to volume, and mix well. Immediately pass the solution through a suitable filter of 0.45-μm pore size, discard the first 5 mL of the filtrate, and use the filtrate for analysis.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector****Assay:** UV 230 nm**Identification test B:** Diode array UV 200–360 nm**Column:** 4.6-mm × 7.5-cm; 3.5-μm packing L1**Column temperature:** 40°**Flow rate:** 1.0 mL/min**Injection volume:** 30 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

 r_u = peak response of oxymorphone from the *Sample solution* r_s = peak response of oxymorphone from the *Standard solution* C_s = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL) C_u = nominal concentration of oxymorphone hydrochloride in the *Sample solution* (mg/mL) M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80 M_{r2} = molecular weight of oxymorphone, 301.34**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS****• DISSOLUTION (711)****Medium:** 0.1 N hydrochloric acid; 900 mL**Apparatus 2:** 50 rpm**Time:** 30 min**Mobile phase:** Dissolve 2.02 g of sodium 1-heptanesulfonate in 800 mL of water and add 200 mL of acetonitrile. Adjust with phosphoric acid to a pH of 2.1.**Standard stock solution:** 0.1 mg/mL of USP Oxymorphone RS in 0.1 N hydrochloric acid**Standard solution:** ($L/1000$) mg/mL of USP Oxymorphone RS in water from the *Standard stock solution*, where L is the label claim in mg/Tablet**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 230 nm**Column:** 4.6-mm × 7.5-cm; 3.5-μm packing L1**Column temperature:** 40°**Flow rate:** 1.0 mL/min**Injection volume:** 60 μL**Run time:** NLT 2.7 times the retention time of oxymorphone**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times V \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

 r_u = peak response of oxymorphone from the *Sample solution* r_s = peak response of oxymorphone from the *Standard solution* C_s = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL) V = volume of *Medium*, 900 mL M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80 M_{r2} = molecular weight of oxymorphone, 301.34 L = label claim (mg/Tablet)**Tolerances:** NLT 80% (Q) of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES**• ORGANIC IMPURITIES**

Protect all solutions containing oxymorphone from light and use clear glass HPLC vials.

Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.**System suitability stock solution A:** 0.2 mg/mL of USP Oxymorphone Related Compound A RS prepared as follows. Transfer an amount of USP Oxymorphone Related Compound A RS to a suitable volumetric flask. Dissolve with 24% of the flask volume of 0.1 N hydrochloric acid and dilute with acetonitrile to volume.**System suitability stock solution B:** 0.02 mg/mL of USP Oxymorphone Related Compound A RS in acetonitrile from *System suitability stock solution A***System suitability stock solution C:** 0.14 mg/mL of USP Oxymorphone RS in *Solution A***System suitability solution:** 0.0008 mg/mL of USP Oxymorphone Related Compound A RS in *System suitability stock solution C* from *System suitability stock solution B***Standard solution:** 0.00014 mg/mL of USP Oxymorphone RS in *Solution A* from *System suitability stock solution C***System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 2 between oxymorphone related compound A and oxymorphone, *System suitability solution***Relative standard deviation:** NMT 10%, *Standard solution***Analysis****Sample:** *Sample solution*

Calculate the percentage of each individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_u/r_T) \times (1/F) \times 100$$

 r_u = peak response of each individual degradation product from the *Sample solution* r_T = sum of peak responses from the *Sample solution* F = relative response factor of each individual degradation product (see *Table 2*)**Acceptance criteria:** See *Table 2*. Disregard any peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
10-Hydroxyoxymorphone ^a	0.58	1.00	0.2
Oxymorphone related compound A (oxymorphone N-oxide)	0.81	1.09	0.2
Oxymorphone	1.00	1.00	—
10-Ketooxymorphone ^b	1.42	0.93	0.2
Oxycodone ^c	2.11	—	—
1-Bromooxymorphone ^{c,d}	2.22	—	—
2,2'-Bisoxymorphone ^e	2.36	1.61	0.2
Any individual unspecified degradation product	—	1.00	0.2
Total degradation products	—	—	1.5

^a 4,5 α -Epoxy-3,10,14-trihydroxy-17-methylmorphinan-6-one.^b 4,5 α -Epoxy-3,14-dihydroxy-17-methylmorphinan-6,10-dione.^c Process impurities, not included in the total degradation products.^d 1-Bromo-4,5 α -epoxy-3,14-dihydroxy-17-methylmorphinan-6-one.^e 2,2'-Bisoxymorphone.**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS (11)**
 - USP Oxymorphone RS
 - USP Oxymorphone Related Compound A RS
 - 4,5 α -Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one N-oxide.
 - C₁₇H₁₉NO₅ 317.34

Oxymorphone Hydrochloride Extended-Release Tablets

DEFINITION

Oxymorphone Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of oxymorphone hydrochloride (C₁₇H₁₉NO₄ · HCl).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B.** The UV absorption spectra of the major peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the Assay.

ASSAY• **PROCEDURE**

Solution A: Dissolve 2.34 g of sodium 1-octanesulfonate monohydrate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.80.

Solution B: Acetonitrile and methanol (50:50)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.00	77.0	23.0
2.50	77.0	23.0

Table 1 (Continued)

Time (min)	Solution A (%)	Solution B (%)
17.50	54.0	46.0
25.00	31.0	69.0
25.05	1.5	98.5
32.50	1.5	98.5
32.55	77.0	23.0
38.00	77.0	23.0

Diluent: Methanol and phosphoric acid (1000:1)

Standard stock solution: 1.78 mg/mL of USP Oxymorphone RS in *Diluent*

Standard solution: 0.357 mg/mL of USP Oxymorphone RS in *Solution A* from the *Standard stock solution*

Sample stock solution: Nominally 2 mg/mL of oxymorphone hydrochloride in *Diluent* prepared as follows. Take NLT 8 Tablets, cut each into small pieces, and transfer to a suitable flask. Add a suitable volume of *Diluent* and shake for at least 16 h. Centrifuge at 3500 rpm for 5 min or until a clear supernatant is obtained.

Sample solution: Nominally 0.4 mg/mL of oxymorphone hydrochloride in *Solution A* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector

Assay: UV 230 nm

Identification test B: Diode array UV 200–360 nm

Column: 4.6-mm × 7.5-cm; 3.5- μ m packing L1

Column temperature: 50°

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of oxymorphone hydrochloride (C₁₇H₁₉NO₄ · HCl) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution*

r_S = peak response of oxymorphone from the *Standard solution*

C_S = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of oxymorphone hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of oxymorphone hydrochloride, 337.80

M_{r2} = molecular weight of oxymorphone, 301.34

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION <711>**

Medium: 45 mM phosphate buffer pH 4.50 (Dissolve 6.16 g of monobasic potassium phosphate in 1 L of water. Adjust with 1 N sodium hydroxide or phosphoric acid to a pH of 4.50); 900 mL

Apparatus 2: 50 rpm, with sinker. [NOTE—The Sotax Helix sinker can be used.]

Time: 1, 2, and 8 h

Mobile phase: Dissolve 1.54 g of ammonium acetate in 925 mL of water and mix well. Add 75 mL of acetonitrile and adjust with trifluoroacetic acid to a pH of 4.50.

Standard stock solution: 0.2 mg/mL of USP Oxymorphone RS in *Medium*
Standard solution: $[(L/900) \times (301.34/337.80)]$ mg/mL of USP Oxymorphone RS in *Medium* from the *Standard stock solution*, where *L* is the label claim in mg/Tablet
Sample solution: Withdraw 1.5 mL of the solution under test

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 230 nm**Column:** 4.6-mm \times 7.5-cm; 4- μ m packing L11**Column temperature:** 60°**Flow rate:** 2.0 mL/min**Injection volume:** 50 μ L**Run time:** NLT 2 times the retention time of oxymorphone**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** 0.8–1.5**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of oxymorphone hydrochloride ($C_{17}H_{19}NO_4 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_i = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_U = peak response of oxymorphone from the *Sample solution**r_S* = peak response of oxymorphone from the *Standard solution**C_S* = concentration of USP Oxymorphone RS in the *Standard solution* (mg/mL)*M_{r1}* = molecular weight of oxymorphone hydrochloride, 337.80*M_{r2}* = molecular weight of oxymorphone, 301.34*V* = volume of *Medium*, 900 mL*L* = label claim (mg/Tablet)**Tolerances:** See *Table 2*.**Table 2**

Time point (i)	Time (h)	Amount Released (%)
1	1	20–40
2	2	35–55
3	8	NLT 80

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Sensitivity solution: 0.357 μ g/mL of USP Oxymorphone RS from the *Standard solution* prepared as follows. Add 20% of the total volume of *Diluent* and dilute with *Solution A* to volume.

System suitability**Samples:** *Standard solution* and *Sensitivity solution***Suitability requirements****Tailing factor:** 0.8–1.5, *Standard solution***Relative standard deviation:** NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*
Analysis

Sample: *Sample solution*

Calculate the percentage of each individual degradation product in the portion of Tablets taken:

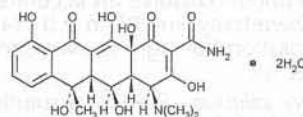
$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution**r_T* = sum of peak responses from the *Sample solution**F* = relative response factor of each individual degradation product (see *Table 3*)**Acceptance criteria:** See *Table 3*. Disregard any peaks less than 0.05%.**Table 3**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxymorphone related compound A ^a (oxymorphone <i>N</i> -oxide)	0.57	1.11	0.2
10-Hydroxyoxymorphone ^b	0.70	1.14	0.2
Oxymorphone	1.00	—	—
10-Ketooxymorphone ^c	1.33	0.97	0.2
Oxycodone ^d	1.82	—	—
14-Hydroxycodeinone ^{d,e}	1.89	—	—
1-Bromooxymorphone ^{d,f}	1.89	—	—
2,2'-Bisoxymorphone ^g	2.28	2.36	0.2
Any individual unspecified degradation product	—	1.00	0.2
Total degradation products	—	—	1.0

^a 4,5 α -Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one *N*-oxide.^b 4,5 α -Epoxy-3,10,14-trihydroxy-17-methylmorphinan-6-one.^c 4,5 α -Epoxy-3,14-dihydroxy-17-methylmorphinan-6,10-dione.^d Process impurities, not included in the total degradation products.^e 4,5 α -Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-7-ene-6-one.^f 1-Bromo-4,5 α -epoxy-3,14-dihydroxy-17-methylmorphinan-6-one.^g 2,2'-Bisoxymorphone.**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS** (11)
USP Oxymorphone RS

Oxytetracycline $C_{22}H_{24}N_2O_9 \cdot 2H_2O$ 496.46

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-, [4S-(4 α ,4a α ,5 α ,5a α ,6 β ,12a α)]-, dihydrate.

4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide dihydrate [6153-64-6].
Anhydrous 460.44 [79-57-2].

» Oxytetracycline has a potency equivalent to not less than 832 μg of $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_9$ per mg.

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards (11)—

USP Endotoxin RS

USP Oxytetracycline RS

Identification—

A: Ultraviolet Absorption (197U)—

Solution: 20 μg per mL.

Medium: 0.1 N hydrochloric acid.

Absorptivity at 353 nm, calculated on the anhydrous basis, is between 96.0% and 104.0% of that of USP Oxytetracycline RS, the potency of the Reference Standard being taken into account.

B: To 1 mg add 2 mL of sulfuric acid: a light red color is produced.

Crystallinity (695): meets the requirements.

pH (791): between 4.5 and 7.0, in an aqueous suspension containing 10 mg per mL.

Water Determination, Method I (921): between 6.0% and 9.0%.

Other requirements—Where the label states that Oxytetracycline is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Oxytetracycline for Injection*. Where the label states that Oxytetracycline must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Oxytetracycline for Injection*.

Assay—

Tetrabutylammonium hydrogen sulfate solution—Dissolve 1 g of tetrabutylammonium hydrogen sulfate in 100 mL of water. Adjust with 1 N sodium hydroxide to a pH of 7.5.

Edetate disodium solution—Dissolve 0.04 g of edetate disodium in 100 mL of water. Adjust with 1 N sodium hydroxide to a pH of 7.5.

pH 7.5 Phosphate buffer—Prepare a mixture of 0.33 M dibasic potassium phosphate and 0.33 M monobasic sodium phosphate (85:15). Adjust, if necessary, by adding more of the appropriate component to a pH of 7.5.

Mobile phase—Transfer, with the aid of 200 mL of water, 50 g of tertiary butyl alcohol to a 1000-mL volumetric flask. Add 60 mL of pH 7.5 Phosphate buffer, 50 mL of Tetrabutylammonium hydrogen sulfate solution, and 10 mL of Edetate disodium solution, and dilute with water to volume. Degas before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Oxytetracycline RS in 0.01 N hydrochloric acid to obtain a solution having a known concentration of about 0.22 mg per mL.

System suitability solution—Prepare a solution of tetracycline hydrochloride in 0.01 N hydrochloric acid containing about 0.2 mg per mL. Mix 3 mL of this solution and 1.5 mL of the *Standard preparation*, and dilute with water to 25 mL.

Assay preparation—Transfer about 44 mg of Oxytetracycline to a 200-mL volumetric flask, add about 25 mL of 0.01 N hydrochloric acid, swirl to dissolve, dilute with 0.01 N hydrochloric acid to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm \times 25-cm column that contains packing L21 and is maintained at $60 \pm 2^\circ$. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for oxytetracycline and 1.0 for tetracycline; and the resolution, R , between the oxytetracycline peak and the tetracycline peak is not less than 5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.25; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in μg , of $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_9$ in each mg of the Oxytetracycline taken by the formula:

$$200(\text{CP} / W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Oxytetracycline RS in the *Standard preparation*; P is the assigned potency, in μg per mg, of USP Oxytetracycline RS; W is the weight, in mg, of the Oxytetracycline taken to prepare the *Assay preparation*; and r_U and r_S are the oxytetracycline peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Oxytetracycline Injection

» Oxytetracycline Injection is a sterile solution of Oxytetracycline with or without one or more suitable anesthetics, antioxidants, buffers, complexing agents, preservatives, and solvents. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of Oxytetracycline ($\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_9$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, protected from light.

USP Reference standards (11)—

USP Endotoxin RS

USP Oxytetracycline RS

Identification—To an accurately measured volume of Injection, equivalent to about 50 mg of oxytetracycline, add 50 mL of methanol, and shake. Using the clear solution so obtained as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

Bacterial Endotoxins Test (85)—It contains not more than 0.4 USP Endotoxin Unit per mg of oxytetracycline.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH (791): between 8.0 and 9.0.

Assay—

Tetrabutylammonium hydrogen sulfate solution, Edetate disodium solution, pH 7.5 Phosphate buffer, Mobile phase, Standard preparation, System suitability solution, and Chromatographic system—Proceed as directed in the *Assay* under *Oxytetracycline*.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of oxytetracycline, to a 500-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Oxytetracycline*. Calculate the quantity, in mg, of $C_{22}H_{24}N_2O_9$ in each mL of the *Injection* taken by the formula:

$$0.5(CP / V)(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Oxytetracycline RS in the *Standard preparation*; *V* is the volume, in mL, of *Injection* taken to prepare the *Assay preparation*; and the other terms are as defined therein.

Oxytetracycline Tablets

» Oxytetracycline Tablets contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{22}H_{24}N_2O_9$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Oxytetracycline RS

Identification—Shake a suitable quantity of finely powdered Tablets with methanol to obtain a solution containing about 1 mg of oxytetracycline per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{22}H_{24}N_2O_9$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 353 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Oxytetracycline RS in the same medium.

Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{22}H_{24}N_2O_9$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Water Determination, Method I (921): not more than 7.5%.

Assay—

Tetrabutylammonium hydrogen sulfate solution, Edetate disodium solution, pH 7.5 Phosphate buffer, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system—Proceed as directed in the *Assay* under *Oxytetracycline*.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of oxytetracycline, to a 500-mL volumetric flask, add about 25 mL of 0.01 N hydrochloric acid, and mix. Dilute with 0.01 N hydrochloric acid to volume, and mix. Filter a portion of this solution through a 0.5- μ m or finer porosity filter, and use the filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Oxytetracycline*. Calculate the quantity, in mg, of $C_{22}H_{24}N_2O_9$ in the portion of Tablets taken by the formula:

$$0.5(CP)(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Oxytetracycline RS in the *Standard preparation*, and the other terms are as defined therein.

Oxytetracycline and Nystatin Capsules

» Oxytetracycline and Nystatin Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$), and not less than 90.0 percent and not more than 135.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Nystatin RS

USP Oxytetracycline RS

Identification—Shake a suitable quantity of Capsule contents with methanol to obtain a solution containing about 1 mg of oxytetracycline per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of oxytetracycline ($C_{22}H_{24}N_2O_9$) dissolved from UV absorbances at the wavelength of maximum absorbance at about 353 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Oxytetracycline RS in the same medium.

Tolerances—Not less than 75% (*Q*) of the labeled amount of $C_{22}H_{24}N_2O_9$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements for *Weight Variation* with respect to oxytetracycline.

Water Determination, Method I (921): not more than 7.5%.

Assay for oxytetracycline—Place not less than 5 Capsules in a high-speed glass blender jar containing an accurately measured volume of 0.1 N hydrochloric acid, and blend for 3 to 5 minutes, so that the stock solution so obtained contains not less than 150 μ g of oxytetracycline ($C_{22}H_{24}N_2O_9$) per mL. Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock solution diluted quantitatively and stepwise with water to yield a *Test Dilution* having a concentration of oxytetracycline assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for nystatin—Proceed as directed for Nystatin under *Antibiotics—Microbial Assays* (81), blending not less than 5 Capsules for 3 to 5 minutes in a high-speed blender with a sufficient accurately measured volume of dimethylformamide to obtain a solution of convenient concentration. Dilute an accurately measured portion of this solution quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Dilute this stock solution quantitatively with ⁶Buffer B.6⁶ (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.

Oxytetracycline and Nystatin for Oral Suspension

» Oxytetracycline and Nystatin for Oral Suspension is a dry mixture of Oxytetracycline and Nystatin with one or more suitable buffers, colors, diluents, flavors, suspending agents, and preservatives. When constituted as directed in the labeling, it contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$), and not less than 90.0 percent and not more than 135.0 percent of the labeled amount of USP Nystatin Units.

Packaging and storage—Preserve in tight, light-resistant containers, at controlled room temperature.

USP Reference standards (11)—

USP Nystatin RS

USP Oxytetracycline RS

Identification—To a quantity of Oxytetracycline and Nystatin for Oral Suspension (powder), equivalent to about 50 mg of oxytetracycline, add 50 mL of methanol, shake, and allow the mixture to settle. Using the clear supernatant as the *Test Solution*, proceed as directed for *Method II* under *Identification*—*Tetracyclines* (193).

Uniformity of dosage units (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements for *Content Uniformity* with respect to oxytetracycline and nystatin.

Deliverable volume (698): meets the requirements.

pH (791): between 4.5 and 7.5, in the suspension constituted as directed in the labeling.

Water Determination, Method I (921): not more than 2.0%.

Assay for oxytetracycline—Constitute Oxytetracycline and Nystatin for Oral Suspension as directed in the labeling. Transfer an accurately measured volume of the suspension so obtained, freshly mixed and free from air bubbles, to a suitable volumetric flask, dilute with 0.1 N hydrochloric acid to volume so that the stock solution so obtained contains not less than 150 µg of oxytetracycline per mL, and mix. Proceed as directed for oxytetracycline under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of the stock solution diluted quantitatively and stepwise with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for nystatin—Constitute Oxytetracycline and Nystatin for Oral Suspension as directed in the labeling. Transfer an accurately measured volume of the suspension so obtained, freshly mixed and free from air bubbles, to a blender jar containing a sufficient, accurately measured volume of dimethylformamide to yield a solution of convenient concentration, and blend at high speed for 3 to 5 minutes. Dilute an accurately measured volume of this solution quantitatively with dimethylformamide to obtain a stock solution containing about 400 USP Nystatin Units per mL. Proceed as directed for nystatin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this solution diluted quantitatively with *Buffer B.6* (CN 1-May-2017) to yield a *Test Dilution* having a concentration of nystatin assumed to be equal to the median dose level of the Standard.

Oxytetracycline Calcium

$C_{44}H_{46}CaN_4O_{18}$ 958.93

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-, calcium salt, [4S-(4α,4aα,5α,5aα,6β,12aα)]-
4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo
2-naphthacenecarboxamide calcium salt [15251-48-6].

» Oxytetracycline Calcium has a potency equivalent to not less than 865 µg of oxytetracycline ($C_{22}H_{24}N_2O_9$) per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store in a cool place.

USP Reference standards (11)—

USP Oxytetracycline RS

Identification—Dissolve a suitable quantity in methanol to obtain a *Test Solution* containing 1 mg of oxytetracycline per mL, and proceed as directed for *Method II* under *Identification*—*Tetracyclines* (193).

Crystallinity (695): meets the requirements.

pH (791): between 6.0 and 8.0, in an aqueous suspension containing 25 mg per mL.

Water Determination, Method I (921): between 8.0% and 14.0%.

Calcium content—Proceed as directed under *Residue on Ignition* (281), except to ignite at $550 \pm 50^\circ$ instead of at $800 \pm 25^\circ$: the weight of residue so obtained, multiplied by 0.2944, gives the equivalent of calcium in the Oxytetracycline Calcium taken. The calcium content is between 3.85% and 4.35%, calculated on the anhydrous basis.

Assay—Dissolve an accurately weighed quantity of Oxytetracycline Calcium in an accurately measured volume of 0.1 N hydrochloric acid to obtain a stock solution having a concentration of about 1000 µg of oxytetracycline per mL. Proceed as directed for oxytetracycline under *Antibiotics—Microbial* (81), using an accurately measured volume of the stock solution diluted quantitatively and stepwise with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Oxytetracycline Calcium Oral Suspension

» Oxytetracycline Calcium Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$). It contains one or more suitable buffers, colors, flavors, preservatives, stabilizers, and suspending agents. In addition, it may contain N-acetylglucosamine.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Oxytetracycline RS

Identification—Shake a suitable quantity of Oral Suspension with methanol to obtain a solution containing 1 mg of oxytetracycline per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed for *Method II* under *Identification*—*Tetracyclines* (193).

Uniformity of dosage units (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698): meets the requirements.

pH (791): between 5.0 and 8.0.

Assay—Transfer an accurately measured quantity of Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 150 mg of oxytetracycline, to a 1000-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix. Proceed as directed for oxytetracycline under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock solution diluted quantitatively and stepwise with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Oxytetracycline Hydrochloride

$C_{22}H_{24}N_2O_9 \cdot HCl$ 496.89

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-, monohydrochloride, [4S-(4 α ,4 α ,5 α ,5 α ,6 β ,12 α)]-
4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [2058-46-0].

» Oxytetracycline Hydrochloride has a potency equivalent to not less than 835 μ g of oxytetracycline ($C_{22}H_{24}N_2O_9$) per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—Where it is intended for use in preparing injectable or ophthalmic dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or ophthalmic dosage forms.

USP Reference standards (11)—

USP Endotoxin RS

USP Oxytetracycline RS

Identification—

A: Ultraviolet Absorption (197U)—

Solution: 20 μ g per mL.

Medium: 0.1 N hydrochloric acid.

Absorptivity, calculated on the dried basis, at 353 nm is between 88.2% and 96.8% of that of USP Oxytetracycline RS, the potency of the Reference Standard being taken into account.

B: To 1 mg add 2 mL of sulfuric acid: a light red color is produced.

Crystallinity (695): meets the requirements.

pH (791): between 2.0 and 3.0, in a solution containing 10 mg per mL.

Loss on drying (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

Other requirements—Where the label states that Oxytetracycline Hydrochloride is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Oxytetracycline for Injection*. Where the label states that Oxytetracycline Hydrochloride must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Oxytetracycline for Injection*. Where it is intended for use in preparing ophthalmic

dosage forms, it is exempt from the requirements for *Bacterial endotoxins*.

Assay—

Tetrabutylammonium hydrogen sulfate solution, Edetate disodium solution, pH 7.5 Phosphate buffer, Mobile phase, Standard preparation, System suitability solution, and Chromatographic system—Proceed as directed in the Assay under *Oxytetracycline*.

Assay preparation—Transfer about 44 mg of Oxytetracycline Hydrochloride to a 200-mL volumetric flask, add about 25 mL of 0.01 N hydrochloric acid, swirl to dissolve, dilute with 0.01 N hydrochloric acid to volume, and mix.

Procedure—Proceed as directed in the Assay under *Oxytetracycline*. Calculate the quantity, in μ g, of oxytetracycline ($C_{22}H_{24}N_2O_9$) in each mg of Oxytetracycline Hydrochloride taken by the formula:

$$200(CP / W)(r_U / r_S)$$

in which the terms are as defined therein.

Oxytetracycline Hydrochloride Capsules

» Oxytetracycline Hydrochloride Capsules contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Oxytetracycline RS

Identification—Shake a suitable quantity of Capsule contents with methanol to obtain a solution containing 1 mg of oxytetracycline per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Procedure—Determine the amount of $C_{22}H_{24}N_2O_9$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 273 nm of filtered portions of the solution under test, suitably diluted with water, in comparison with a Standard solution having a known concentration of USP Oxytetracycline RS in the same medium, using 5 mL of 0.1 N hydrochloric acid to dissolve the Standard.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{22}H_{24}N_2O_9$ is dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements.

Loss on drying (731)—Dry about 100 mg of Capsule contents, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.

Assay—

Tetrabutylammonium hydrogen sulfate solution, Edetate disodium solution, pH 7.5 Phosphate buffer, Mobile phase, Standard preparation, Resolution solution, and Chromatographic system—Proceed as directed in the Assay under *Oxytetracycline*.

Assay preparation—Remove, as completely as possible, the contents of not less than 20 Capsules, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of oxytetracycline, to a 500-mL volumetric flask, add about 50 mL of 0.01 N hydrochloric acid, and

swirl to dissolve. Dilute with 0.01 N hydrochloric acid to volume, mix, and filter a portion of the solution through a 0.5- μ m or finer porosity filter. Use the filtrate as the Assay preparation.

Procedure—Proceed as directed for *Procedure* in the Assay under Oxytetracycline. Calculate the quantity, in mg, of oxytetracycline ($C_{22}H_{24}N_2O_9$) in the portion of Capsules taken by the formula:

$$0.5(CP)(r_U / r_S)$$

in which the terms are as defined therein.

Oxytetracycline for Injection

» Oxytetracycline for Injection contains an amount of Oxytetracycline Hydrochloride equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$).

Change to read:

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017), protected from light.

USP Reference standards (11)—

USP Endotoxin RS

USP Oxytetracycline RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

Bacterial Endotoxins Test (85)—It contains not more than 0.4 USP Endotoxin Unit per mg of oxytetracycline.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined, Fluid D* being used instead of *Fluid A*.

pH (791): between 1.8 and 2.8, in a solution containing 25 mg per mL.

Loss on drying (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.0% of its weight.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Other requirements—It responds to *Identification test B* under Oxytetracycline Hydrochloride. It also meets the requirements for *Uniformity of Dosage Units* (905) and *Labeling* (7), *Labels and Labeling for Injectable Products*.

Assay—

Tetrabutylammonium hydrogen sulfate solution, Edetate disodium solution, pH 7.5 Phosphate buffer, Mobile phase, Standard preparation, System suitability solution, and Chromatographic system—Proceed as directed in the Assay under Oxytetracycline.

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Oxytetracycline for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.2 mg of oxytetracycline per mL.

Assay preparation 2 (where the label states the quantity of oxytetracycline in a given volume of constituted solu-

tion)—Constitute Oxytetracycline for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.2 mg of oxytetracycline per mL.

Procedure—Proceed as directed for *Procedure* in the Assay under Oxytetracycline. Calculate the quantity, in mg, of oxytetracycline ($C_{22}H_{24}N_2O_9$) withdrawn from the container or in the portion of constituted solution taken by the formula:

$$(L / D)(CP)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of oxytetracycline ($C_{22}H_{24}N_2O_9$) in the container or in the portion of constituted solution taken; *D* is the concentration, in mg per mL, of oxytetracycline in *Assay preparation 1* or in *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution; and the other terms are as defined therein.

Oxytetracycline Hydrochloride Soluble Powder

» Oxytetracycline Hydrochloride Soluble Powder is a dry mixture of Oxytetracycline Hydrochloride and one or more suitable excipients. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of oxytetracycline hydrochloride ($C_{22}H_{24}N_2O_9 \cdot HCl$).

Packaging and storage—Preserve in well-closed containers.

Labeling—Label it to indicate that it is for oral veterinary use only.

USP Reference standards (11)—

USP Oxytetracycline RS

Identification—

A: Shake a quantity of Soluble Powder with methanol to obtain a solution containing about 1 mg of oxytetracycline hydrochloride per mL. Filter if necessary to obtain a clear solution. Using the filtrate as the *Test solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

pH (791): between 1.5 and 3.0, in the solution obtained as directed in the labeling.

Loss on drying (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.0% of its weight.

Minimum fill (755): meets the requirements.

Assay—

Tetrabutylammonium hydrogen sulfate solution, Edetate disodium solution, pH 7.5 Phosphate buffer, Mobile phase, Standard preparation, System suitability solution, and Chromatographic system—Proceed as directed in the Assay under Oxytetracycline.

Assay preparation—Transfer an accurately weighed portion of the Soluble Powder, equivalent to about 100 mg of oxytetracycline hydrochloride, to a 500-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Pass a portion of this solution through a filter having a

0.5- μ m or finer porosity. Use the filtrate as the Assay preparation.

Procedure—Proceed as directed for Procedure in the Assay under Oxytetracycline. Calculate the quantity, in g, of oxytetracycline hydrochloride ($C_{22}H_{24}N_2O_9 \cdot HCl$) in each g of Soluble Powder taken by the formula:

$$0.5(496.90 / 460.44)(CP / W)(r_u / r_s)$$

in which 496.90 and 460.44 are the molecular weights of oxytetracycline hydrochloride and oxytetracycline, respectively; C is the concentration, in mg per mL, of USP Oxytetracycline RS in the Standard preparation; P is the assigned potency, in μ g of oxytetracycline per mg, of USP Oxytetracycline RS; W is the weight, in g, of Soluble Powder taken to prepare the Assay preparation; and r_u and r_s are the oxytetracycline peak responses obtained from the Assay preparation and the Standard preparation, respectively.

Oxytetracycline Hydrochloride and Hydrocortisone Acetate Ophthalmic Suspension

» Oxytetracycline Hydrochloride and Hydrocortisone Acetate Ophthalmic Suspension is a sterile suspension of Oxytetracycline Hydrochloride and Hydrocortisone Acetate in a suitable oil vehicle with one or more suitable suspending agents. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in tight, light-resistant containers. The containers are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards (11)—

USP Hydrocortisone Acetate RS

USP Oxytetracycline RS

Sterility Tests (71)—It meets the requirements when tested as directed for Direct Inoculation of the Culture Medium under Test for Sterility of the Product to be Examined using 0.25 mL of specimen.

Water Determination, Method I (921): not more than 1.0%, 60 mL of a mixture of methanol and chloroform (2:1) being used instead of methanol in the titration vessel.

Assay for oxytetracycline—Transfer an accurately measured volume of Ophthalmic Suspension to a separator, add 50 mL of ether, and shake. Add 25 mL of 0.1 N hydrochloric acid, shake, and allow to separate. Collect the acid layer, and repeat the extraction with three additional 25-mL portions of 0.1 N hydrochloric acid. Combine the acid extracts in a 200-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix. Proceed as directed for oxytetracycline under Antibiotics—Microbial Assays (81), using an accurately measured volume of this solution diluted quantitatively and stepwise with water to obtain a Test Dilution having a concentration assumed to be equal to the median dose level of the Standard.

Assay for hydrocortisone acetate—

Mobile phase—Prepare a degassed and filtered mixture of water and methanol (50:50).

Standard preparation—Dissolve an accurately weighed quantity of USP Hydrocortisone Acetate RS in a mixture of

Mobile phase and alcohol (80:20) to obtain a solution having a known concentration of about 0.06 mg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Suspension, equivalent to about 30 mg of hydrocortisone acetate, to a separator containing 25 mL of pH 9.0 alkaline borate buffer (see under Buffer Solutions in the section Reagents, Indicators, and Solutions). Extract with four 25-mL portions of chloroform, filtering each chloroform extract through a thin layer of chloroform-washed anhydrous sodium sulfate into a 250-mL volumetric flask. Rinse the sodium sulfate with chloroform, collecting the filtrate in the volumetric flask, dilute with chloroform to volume, and mix. Transfer 25.0 mL of the resulting solution to a 50-mL conical flask, and evaporate slowly with the aid of mild heat until about 5 mL remains. Add about 15 mL of alcohol, and evaporate slowly until about 5 mL remains. Transfer this solution to a 50-mL volumetric flask, dilute with a mixture of Mobile phase and alcohol (80:20) to volume, and mix.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm \times 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the column efficiency determined from the analyte peak is not less than 235 theoretical plates, the tailing factor for the analyte peak is not more than 1.7, and the relative standard deviation of replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 μ L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{23}H_{32}O_6$ in each mL of the Ophthalmic Suspension taken by the formula:

$$500(C / V)(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone Acetate RS in the Standard preparation; V is the volume, in mL, of Ophthalmic Suspension taken; and r_u and r_s are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

Oxytetracycline Hydrochloride and Hydrocortisone Ointment

» Oxytetracycline Hydrochloride and Hydrocortisone Ointment contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone.

Packaging and storage—Preserve in collapsible tubes or in well-closed, light-resistant containers.

USP Reference standards (11)—

USP Hydrocortisone RS

USP Oxytetracycline RS

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for oxytetracycline—Transfer a suitable, accurately weighed quantity of Ointment to a separator, add 50 mL of ether, and shake. Add 20 mL of 0.1 N hydrochloric acid, shake, and allow to separate. Collect the acid layer, and repeat the extraction with three additional 20-mL portions

of 0.1 N hydrochloric acid. Combine the acid extracts in a 100-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix. Quantitatively dilute a portion of this solution with 0.1 N hydrochloric acid so that the solution so obtained contains not less than 150 µg of oxytetracycline per mL. Proceed as directed for oxytetracycline under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this solution diluted quantitatively and stepwise with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for hydrocortisone—Proceed with Ointment as directed in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc, and Hydrocortisone Ophthalmic Ointment*.

Oxytetracycline Hydrochloride and Polymyxin B Sulfate Ointment

» Oxytetracycline Hydrochloride and Polymyxin B Sulfate Ointment contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$), and not less than 90.0 percent and not more than 125.0 percent of the labeled amount of polymyxin B.

Packaging and storage—Preserve in collapsible tubes, or in well-closed, light-resistant containers.

USP Reference standards (11)—

USP Oxytetracycline RS
USP Polymyxin B Sulfate RS

Minimum fill (755): meets the requirements.

Water Determination, Method I (921): not more than 1.0%, 10 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for oxytetracycline—Proceed with Ointment as directed in the *Assay for oxytetracycline* under *Oxytetracycline Hydrochloride and Hydrocortisone Ointment*.

Change to read:

Assay for polymyxin B—Transfer an accurately weighed quantity of Ointment, equivalent to about 10,000 USP Polymyxin B Units, to a 15-mL centrifuge tube, add 10 mL of ether, stir, and centrifuge for 10 minutes. Decant and discard the clear ether. Wash the residue with 10 mL of ether, centrifuge for 10 minutes, decanting and discarding the clear ether. Wash the residue with several 10-mL portions of acetone, centrifuging, decanting, and discarding each washing until the yellow color is removed from the residue. [NOTE—Take care not to remove any of the residue with the washings.] Add 0.2 mL of polysorbate 80 to the residue, and mix. Transfer the mixture to a 100-mL volumetric flask with the aid of *Buffer B.6* (CN 1-May-2017), dilute with the same solvent to volume, and mix. Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this solution diluted quantitatively with *Buffer B.6* (CN 1-May-2017) to yield a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.

Oxytetracycline Hydrochloride and Polymyxin B Sulfate Ophthalmic Ointment

DEFINITION

Oxytetracycline Hydrochloride and Polymyxin B Sulfate Ophthalmic Ointment is a sterile ointment containing Oxytetracycline Hydrochloride and Polymyxin B Sulfate. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of oxytetracycline and NLT 90.0% and NMT 125.0% of the labeled amount of polymyxin B.

ASSAY

• OXYTETRACYCLINE

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Transfer a suitable, weighed quantity of the Ophthalmic Ointment to a separator, add 50 mL of ether, and shake. Add 20 mL of 0.1 N hydrochloric acid, shake, and allow to separate. Collect the acid layer, and repeat the extraction with three additional 20-mL portions of 0.1 N hydrochloric acid. Combine the acid extracts in a 100-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume. Dilute a portion of this solution with 0.1 N hydrochloric acid to obtain a solution containing NLT 150 µg/mL of oxytetracycline.

Analysis: Proceed as directed in the chapter, using a suitable aliquot of the *Sample solution* diluted with water, to yield a *Test Dilution* having an oxytetracycline concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–120.0%

• POLYMYXIN B

(See *Antibiotics—Microbial Assays* (81).)

Sample solution: Transfer nominally 10,000 USP Polymyxin B Units from the Ophthalmic Ointment to a 15-mL centrifuge tube. Add 10 mL of ether, stir, and centrifuge for 10 min. Decant, and discard the clear ether. Wash the residue with 10 mL of ether, and centrifuge for 10 min, decanting and discarding the clear ether. Wash the residue with several 10-mL portions of acetone, centrifuging, decanting, and discarding each washing until the yellow color is removed from the residue. Take care not to remove any of the residue with the washings. Add 0.2 mL of polysorbate 80 to the residue, and mix. Transfer the mixture to a 100-mL volumetric flask with the aid of *Buffer B.6*, and dilute with the same solvent to volume.

Analysis: Proceed as directed in the chapter, using a suitable aliquot of the *Sample solution* diluted with *Buffer B.6*, to yield a *Test Dilution* having a polymyxin B concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–125.0%

SPECIFIC TESTS

- **STERILITY TESTS** (71): Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Oxytetracycline RS
USP Polymyxin B Sulfate RS

Oxytetracycline Hydrochloride and Polymyxin B Sulfate Topical Powder

» Oxytetracycline Hydrochloride and Polymyxin B Sulfate Topical Powder contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amounts of oxytetracycline ($C_{22}H_{24}N_2O_9$) and polymyxin B in a suitable, fine powder base.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Oxytetracycline RS
USP Polymyxin B Sulfate RS

Minimum fill (755): meets the requirements.

Loss on drying (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

Assay for oxytetracycline—Transfer a suitable, accurately weighed quantity of Topical Powder to a glass blender jar containing a sufficient, accurately measured volume of 0.1 N hydrochloric acid to yield a stock solution containing not less than 150 µg of oxytetracycline per mL, and blend at high speed for 3 to 5 minutes. Proceed as directed for oxytetracycline under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this solution diluted quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration of oxytetracycline assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for polymyxin B—Transfer an accurately weighed quantity of Topical Powder, equivalent to about 10,000 USP Polymyxin B Units, to a 50-mL centrifuge tube, add 15 mL of acetone and 0.05 mL of hydrochloric acid, and stir. Add 20 mL of acetone, and centrifuge for 10 minutes. Decant and discard the clear liquid. Add 15 mL of acetone and 0.05 mL of hydrochloric acid to the residue, stir, add 20 mL of acetone, and centrifuge for 10 minutes, decanting and discarding the clear liquid. [NOTE—Take care not to discard any of the residue with the clear liquid.] Add 5 mL of *Buffer B.6* (CN 1-May-2017) to the residue, and mix. Transfer the mixture to a 100-mL volumetric flask with the aid of *Buffer B.6* (CN 1-May-2017), dilute with the same solvent to volume, and mix. Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock solution diluted quantitatively with *Buffer B.6* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.

Oxytetracycline Hydrochloride and Polymyxin B Sulfate Vaginal Inserts

» Oxytetracycline Hydrochloride and Polymyxin B Sulfate Vaginal Inserts contain the equivalent of

not less than 90.0 percent and not more than 120.0 percent of the labeled amounts of oxytetracycline ($C_{22}H_{24}N_2O_9$) and polymyxin B.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Oxytetracycline RS
USP Polymyxin B Sulfate RS

Loss on drying (731)—Dry about 100 mg, accurately weighed, of powdered Vaginal Inserts in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.0% of its weight.

Assay for oxytetracycline—Place not fewer than 5 Vaginal Inserts in a high-speed blender jar containing an accurately measured volume of 0.1 N hydrochloric acid, so that the stock solution obtained after blending for 3 to 5 minutes contains not less than 150 µg of oxytetracycline per mL. Proceed as directed for oxytetracycline under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this solution diluted quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration of oxytetracycline assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for polymyxin B—Weigh and finely powder not fewer than 5 Vaginal Inserts. Transfer an accurately weighed portion of the powder, equivalent to about 100,000 USP Polymyxin B Units, to a filter funnel equipped with a solvent-resistant membrane filter (1-µm or finer porosity). Wash the powder with five 20-mL portions of acetone, applying vacuum, and discarding the accumulated filtrate. [NOTE—If necessary, wash the powder with additional portions of acetone to remove any yellow color.] Carefully transfer the filter and the washed powder to a beaker containing about 400 mL of *Buffer B.6* (see *Antibiotics—Microbial Assays* (81), *Media and Solutions, Solutions, Buffers*), (CN 1-May-2017) and stir. Transfer the contents of the beaker to a 500-mL volumetric flask with the aid of *Buffer B.6* (CN 1-May-2017), dilute with the same solvent to volume, and mix. Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this solution diluted quantitatively and stepwise with *Buffer B.6* (CN 1-May-2017) to yield a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.

Oxytocin



1007.19

Oxytocin.

Oxytocin [50-56-6].

» Oxytocin is a nonapeptide hormone having the property of causing the contraction of uterine smooth muscle and of the myoepithelial cells within the mammary gland. It is prepared by synthesis. Its oxytocic activity is not less than 400 USP Oxytocin Units per mg.

Packaging and storage—Preserve in tight containers, preferably of Type I glass, in a refrigerator.

USP Reference standards (11)—

USP Oxytocin RS

USP Oxytocin Identification RS

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—The total bacterial count does not exceed 200 cfu per g. For products of animal origin, it meets also the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

Identification—

A: The retention time of the oxytocin peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

Perform one of the following two tests:

B: Nuclear Magnetic Resonance—[NOTE—Concentrations of Oxytocin in both the *Standard solution* and the *Test solution* must be the same (within 5% of each other) but can be adjusted based on the quality of the spectrum obtained. The spectra must be acquired under the same conditions for both the *Standard solution* and the *Test solution*. The spectra obtained are of sufficient quality to allow quantification of the integrals of the resonances specified below to be obtained. Integrals and spectra of both the *Standard solution* and the *Test solution* can be repeated and averaged.]

pH 5.0 Sodium phosphate buffer—Dissolve 27.6 g of monobasic sodium phosphate in 900 mL of water, adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 5.0 ± 0.1, dilute with water to 1000 mL, and mix.

Standard solution—Prepare a 10 mg per mL solution (approximately 1 mL) of USP Oxytocin Identification RS in pH 5.0 Sodium phosphate buffer. Lyophilize to dryness, redissolve in deuterium oxide, lyophilize again, redissolve in deuterium oxide, and lyophilize once again (to replace exchangeable hydrogens with deuterium). Dissolve in 1 mL of deuterium oxide containing 0.5% v/v (2,2,3,3-d₄)-3-(trimethylsilyl) propionic acid sodium salt (TSP) as a chemical shift reference.

Test solution—Prepare a 10 mg per mL solution (approximately 1 mL) of Oxytocin in pH 5.0 Sodium phosphate buffer. Proceed as directed for the *Standard solution*.

Procedure—Obtain a proton NMR spectrum of both the *Standard solution* and the *Test solution*. The spectra from both solutions are qualitatively and quantitatively similar, and all the resonances from the spectrum of the *Standard solution* are present in the spectrum of the *Test solution* and have the same chemical shift values (±0.1 ppm). Identify any other resonances in the spectrum of the *Test solution* that are not present in the spectrum of the *Standard solution*. The integrals of the acetate and deuterium oxide peaks at 1.9 ppm and 4.9 ppm can differ quantitatively in the spectra of the *Standard solution* and the *Test solution*.

C: Amino acid content—Use a suitable, validated procedure (see *Biotechnology-Derived Articles—Amino Acid Analysis* (1052)).

Standard solutions—Prepare a solution having known equimolar amounts of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine with half the equimolar amount of L-cystine. For the validation of the method, use an appropriate internal standard, such as norleucine. Prepare a separate, equimolar solution of L-tryptophan.

Test solution—[NOTE—The following hydrolysis conditions and concentrations can be modified depending on the method of analysis chosen.] Transfer about 64 mg of Oxytocin, accurately weighed, to a suitable vessel, and dissolve in 1.0 mL of water. Transfer 0.10 mL of this solution to a vacuum hydrolysis tube, add 2.0 mL of 6 N hydrochloric acid, evacuate the tube, and heat for 16 hours at 120°. Transfer 0.10 mL of the hydrolysate so obtained to a suitable vessel, add 1 mL of water, and lyophilize. Dissolve in and dilute to

a suitable volume in a buffer solution suitable for amino acid analysis.

Procedure—Inject equal volumes of the *Standard solutions* and the *Test solution* into the amino acid analyzer, and measure and record the responses for each amino acid peak. Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking 1/6 of the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, isoleucine, and leucine as equal to 1. The values fall within the following limits: aspartic acid: 0.90 to 1.10; glutamic acid: 0.90 to 1.10; proline: 0.90 to 1.10; glycine: 0.90 to 1.10; leucine: 0.90 to 1.10; isoleucine: 0.90 to 1.10; tyrosine: 0.7 to 1.05; half-cystine: 1.4 to 2.1. Not more than traces of other amino acids are present.

Acetic acid content (503): between 6% and 10%.

Test Solution—Transfer about 15 mg of Oxytocin, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Ordinary impurities—The sum of the responses of impurities in the chromatogram of the *Assay preparation* obtained in the *Assay* is not more than 5% of the area of the oxytocin peak.

Assay—

Mobile phase A—Prepare a buffer solution of 0.1 M monobasic sodium phosphate.

Mobile phase B—Prepare a filtered and degassed mixture of acetonitrile in water (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Diluent—Dissolve 5.0 g of chlorobutanol in 5.0 mL of glacial acetic acid, add 5.0 g of alcohol, 1.1 g of sodium acetate, and 1000 mL of water, and mix.

Standard preparation—Dissolve the entire contents of a vial of USP Oxytocin RS in a known volume of *Diluent*.

[NOTE—The solution may be diluted as necessary to a working concentration range for the assay.]

Assay preparation—Dissolve an accurately weighed quantity of Oxytocin in *Diluent* to obtain a solution containing about 10 USP Oxytocin Units per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a variable wavelength detector set at 220 nm and a 12.0-cm × 4.6-mm column that contains 5-μm packing L1, and is programmed to provide variable mixtures of *Mobile phase A* and *Mobile phase B*. The column is maintained at room temperature, and the flow rate is about 1.5 mL per minute. The system is equilibrated with a mixture of 70% *Mobile phase A* and 30% *Mobile phase B*. After each injection of the *Standard preparation* and the *Assay preparation*, the composition of the mobile phase is changed linearly over the next 20 minutes so that it consists of a mixture of 50% *Mobile phase A* and 50% *Mobile phase B*. Chromatograph the *Standard preparation*, and record the chromatograms as directed for *Procedure*. Adjust the flow rate or the composition of the *Mobile phase* such that the retention time of oxytocin is approximately 10 minutes and between 15 and 17 minutes for chlorobutanol. The resolution, *R*, between oxytocin and the nearest adjacent peak is not less than 1.5, and the relative standard deviation for replicate injections is not more than 2.0% for oxytocin.

Procedure—Separately inject three equal volumes (about 100 μL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, and record the chromatograms as described under *Chromatographic system*. Identify the peaks, and determine the area of the oxytocin peak. Calculate the potency of oxytocin in USP Oxytocin Units per mg by the formula:

$$C(r_u/r_s)(V/W)$$

in which *C* is the concentration, in USP Oxytocin Units per mL, of the *Standard preparation*; and *r_u* and *r_s* are the mean

peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively; V is the volume of sample solution in which the sample was dissolved; and W is the amount, in mg, of oxytocin dissolved in the sample solution.

Oxytocin Injection

» Oxytocin Injection is a sterile solution of Oxytocin in a suitable diluent. Each mL of Oxytocin Injection possesses an oxytocic activity of not less than 90.0 percent and not more than 110.0 percent of that stated on the label in USP Oxytocin Units.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass or in suitable plastic containers.

Labeling—Label it to indicate its oxytocic activity in USP Oxytocin Units per mL. Label it also to state the animal source if naturally derived, or to state that it is synthetic.

USP Reference standards (11)—

USP Endotoxin RS

USP Oxytocin RS

Bacterial Endotoxins Test (85)—It contains not more than 35.7 Endotoxin Units per USP Oxytocin Unit.

pH (791): between 3.0 and 5.0.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

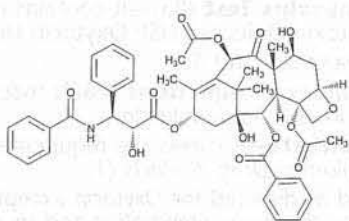
Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—Proceed as directed for Oxytocin except to use undiluted Injection as the *Assay preparation* and to allow not less than 25 minutes between injections. Calculate the potency, in USP Oxytocin Units per mL, by the formula:

$$C(r_u / r_s)$$

in which C is the concentration, in USP Oxytocin Units per mL, of the *Standard preparation*; and r_u and r_s are the mean values of the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Paclitaxel



$C_{47}H_{51}NO_{14}$ 853.91

Benzenepropanoic acid, β -(benzoylamino)- α -hydroxy-, 6,12-bis(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxet-9-yl ester, [2aR-[2a α ,4 β ,4a β ,6 β ,9 α (α R*, β S*),11 α ,12 α ,12a α ,12b α]]-, (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-1,2a,3,4,4a,6,9,10,11,12,12a,12b-Dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5H-cyclodeca[3,4]-benz[1,2-b]oxet-5-one 6,12b-diacetate, 12-benzoate, 9-ester with (2R,3S)-N-benzoyl-3-phenylisoserine [33069-62-4].

» Paclitaxel contains not less than 97.0 percent and not more than 102.0 percent of $C_{47}H_{51}NO_{14}$, calculated on the anhydrous, solvent-free basis.

Caution—Paclitaxel is cytotoxic. Great care should be taken to prevent inhaling particles of Paclitaxel and exposing the skin to it.

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.

Labeling—The labeling indicates the type of process used to produce the material and the *Related compounds* test with which the material complies.

USP Reference standards (11)—

USP Endotoxin RS

USP Paclitaxel RS

USP Paclitaxel Related Compound A RS

Cephalomannine.

USP Paclitaxel Related Compound B RS

10-Deacetyl-7-epipaclitaxel.

USP Paclitaxel Impurity Mixture RS

Mixture of paclitaxel and the following related compounds: propyl analog, cephalomannine, *sec*-butyl analog, *n*-butyl analog, benzyl analog, baccatin VI, pentyl analog, and 7-epipaclitaxel.

Identification—

A: *Infrared Absorption* (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Specific rotation (781S): between -49.0° and -55.0° at 20° , calculated on the anhydrous, solvent-free basis.

Test solution: 10 mg per mL, in methanol.

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—The total aerobic microbial count does not exceed 100 cfu per g. It meets the requirements of the tests for the absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* species, and *Escherichia coli*.

Bacterial Endotoxins Test (85)—It contains not more than 0.4 USP Endotoxin Unit per mg of paclitaxel.

Water Determination, Method 1c (921): not more than 4.0%.

Residue on ignition (281): not more than 0.2%.

Delete the following:

• **Heavy metals, Method II** (231): 0.002%. • (Official 1-Jan-2018)

Related compounds—

TEST 1 (FOR MATERIAL LABELED AS ISOLATED FROM NATURAL SOURCES)—If the material complies with this test, the labeling indicates that it meets USP *Related compounds Test 1*.

Diluent—Prepare as directed in the *Assay*.

Solution A—Prepare filtered and degassed acetonitrile.

Solution B—Prepare filtered and degassed water.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability solution—Dissolve accurately weighed quantities of USP Paclitaxel Related Compound A RS and USP Paclitaxel Related Compound B RS in methanol to obtain a solution having known concentrations of about 10 μ g of each per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Standard solution—Dissolve, with the aid of sonication, an accurately weighed quantity of USP Paclitaxel RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 5 μ g per mL.

Test solution—Use the *Assay preparation*.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm \times 25-cm column that contains 5- μ m packing L43. The flow rate is about 2.6 mL per minute. The column temperature is maintained at 30° . The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–35	35	65	isocratic
35–60	35→80	65→20	linear gradient
60–70	80→35	20→65	linear gradient
70–80	35	65	isocratic

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.78 for paclitaxel related compound A and 0.86 for paclitaxel related compound B (relative to the retention time for paclitaxel obtained from the *Test solution*); and the resolution, *R*, between paclitaxel related compound A and paclitaxel related compound B is not less than 1.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject a volume (about 15 μ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for the major peaks. Calculate the percentage of each impurity in the portion of Paclitaxel taken by the formula:

$$100(F_i / r_u)$$

in which *F* is the relative response factor for each impurity peak (see *Table 1* for values); *r_i* is the peak area for each individual impurity; and *r_u* is the peak area for paclitaxel.

Table 1

Relative Retention Time	Relative Response Factor (F)	Name	Limit (%)
0.24	1.29	Baccatin III	0.2
0.53	1.00	10-Deacetylpaclitaxel	0.5
0.57	1.00	7-Xylosylpaclitaxel	0.2
0.78	1.26	Cephalomannine (paclitaxel related compound A)	a ₁ ¹
0.78	1.26	2'',3''-Dihydrocephalomanine	a ₂ ¹
0.86	1.00	10-Deacetyl-7-epipaclitaxel (paclitaxel related compound B)	0.5
1.10	1.00	Benzyl analog ³	b ₁ ²
1.10	1.00	3'',4''-Dehydropaclitaxel C	b ₂ ²
1.40	1.00	7-Epicephalomannine	0.3
1.85	1.00	7-Epipaclitaxel	0.5

¹ Resolution may be incomplete for these peaks, depending upon the relative amounts present; the sum of a₁ and a₂ is not more than 0.5%.

² Resolution may be incomplete for these peaks, depending upon the relative amounts present; the sum of b₁ and b₂ is not more than 0.5%.

³ The following chemical name is assigned to the related compound, benzyl analog: Baccatin III 13-ester with (2*R*,3*S*)-2-hydroxy-3-phenyl-3-(2-phenylacetylaminopropanoic acid.

In addition to not exceeding the limits for paclitaxel related impurities in Table 1, not more than 0.1% of any other single impurity is found; and not more than 2.0% of total impurities is found.

TEST 2 (FOR MATERIAL LABELED AS PRODUCED BY A SEMISYNTHETIC PROCESS)—If the material complies with this test, the labeling indicates that it meets USP *Related compounds* Test 2.

Diluent—Use acetonitrile.

Solution A—Use a filtered and degassed mixture of water and acetonitrile (3:2).

Solution B—Use filtered and degassed acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability solution—Dissolve accurately weighed quantities of USP Paclitaxel RS and USP Paclitaxel Related Compound B RS in *Diluent*, shaking and sonicating if necessary, to obtain a solution having known concentrations of about 0.96 mg and 0.008 mg per mL, respectively.

Test solution—Transfer about 10 mg of Paclitaxel, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, shaking and sonicating if necessary, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm × 15-cm column that contains 3-μm packing L1. The flow rate is about 1.2 mL per minute. The column temperature is maintained at 35°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–20	100	0	isocratic
20–60	100→10	0→90	linear gradient
60–62	10→100	90→0	linear gradient
62–70	100	0	isocratic

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.94 for paclitaxel related compound B and 1.0 for paclitaxel; the resolution, *R*, between paclitaxel related compound B and paclitaxel is not less than

1.2; and the relative standard deviation for replicate injections is not more than 2.0% for the paclitaxel peak.

Procedure—Separately inject equal volumes (about 15 μL) of the *Diluent* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Disregard any peaks due to the *Diluent*. Calculate the percentage of each impurity in the portion of Paclitaxel taken by the formula:

$$100(F_i / r_s)$$

in which *F* is the relative response factor for each impurity (see Table 2 for values); *r_i* is the peak area for each impurity obtained from the *Test solution*; and *r_s* is the sum of the areas of all the peaks obtained from the *Test solution*.

Table 2

Relative Retention Time	Relative Response Factor (F)	Name	Limit (%)
0.11	1.24	10-Deacetylbaaccatin III	0.1
0.20	1.29	Baccatin III	0.2
0.42	1.39	Photodegradant ²	0.1
0.47	1.00	10-Deacetylpaclitaxel	0.5
0.80	1.00	2-Debenzoypaclitaxel-2-pentenoate	0.7
0.92 ¹	1.00	Oxetane ring opened, acetyl and benzoyl migrated ²	x ₁
0.92 ¹	1.00	10-Acetoacetylpaclitaxel	x ₂
0.94 ¹	1.00	10-Deacetyl-7-epipaclitaxel (paclitaxel related compound B)	x ₃
1.37	1.00	7-Epipaclitaxel	0.4
1.45	1.00	10,13-Bissidechainpaclitaxel ²	0.5
1.54	1.00	7-Acetylpaclitaxel	0.6
1.80	1.75	13-Tes-baccatin III	0.1
2.14	1.00	7-Tes-paclitaxel	0.3

¹ Resolution may be incomplete for these peaks, depending upon the relative amounts present; the sum of x₁, x₂, and x₃ is not more than 0.4%.

² The following chemical names are assigned to the related compounds Photodegradant; Oxetane ring opened, acetyl and benzoyl migrated; and 10,13-Bissidechainpaclitaxel:

Photodegradant
(1*R*,2*R*,4*S*,5*S*,7*R*,10*S*,11*R*,12*S*,13*S*,15*S*,16*S*)-2,10-diacetyloxy-5,13-dihydroxy-4,16,17,17-tetramethyl-8-oxa-3-oxo-12-phenylcarbonyloxypentacyclo[11.3.1.0^{1,11}.0^{4,11}.0^{7,10}]heptadec-15-yl(2*R*,3*S*)-2-hydroxy-3-phenyl-3-(phenylcarbonylamino)propanoate
Oxetane ring opened, acetyl and benzoyl migrated
(1*S*,2*S*,3*R*,4*S*,5*S*,7*S*,8*S*,10*R*,13*S*)-5,10-diacetyloxy-1,2,4,7-tetrahydroxy-8,12,15,15-tetramethyl-9-oxo-4-(phenylcarbonyloxymethyl)tricyclo[9.3.1.0^{3,8}]pentadec-11-en-13-yl(2*R*,3*S*)-2-hydroxy-3-phenyl-3-(phenylcarbonylamino)propanoate
10,13-Bissidechainpaclitaxel
Baccatin III 13-ester with (2*R*,3*S*)-2-hydroxy-3-phenyl-3-(phenylcarbonylamino)propanoic acid, 10-ester with (2*S*,3*S*)-2-hydroxy-3-phenyl-3-(phenylcarbonylamino)propanoic acid

In addition to not exceeding the limits for paclitaxel related impurities in Table 2, not more than 0.1% of any other single impurity is found; and not more than 2.0% of total impurities is found.

TEST 3 (FOR MATERIAL LABELED AS PRODUCED BY A PLANT CELL FERMENTATION PROCESS)—If the material complies with this test, the labeling indicates that it meets USP *Related compounds* Test 3.

Solution A—Prepare a filtered and degassed mixture of water and acetonitrile (3:2).

Solution B—Prepare filtered and degassed acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability solution—Dissolve USP Paclitaxel Impurity Mixture RS in acetonitrile, sonicating if necessary, to obtain a solution having a known concentration of about 1 mg per mL.

Standard solution—Dissolve an accurately weighed quantity of USP Paclitaxel RS in acetonitrile, sonicating if necessary, to obtain a solution having a known concentration of about 1 mg per mL.

Test solution—Transfer about 10 mg of Paclitaxel, accurately weighed, to a 10-mL volumetric flask. Dissolve in and dilute with acetonitrile to volume, sonicating if necessary, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm × 15-cm column that contains 3-μm packing L1. The flow rate is about 1.2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–28	100	0	isocratic
28–33	100→98	0→2	linear gradient
33–58	98→10	2→90	linear gradient
58–60	10	90	isocratic
60–63	10→100	90→0	linear gradient
63–70	100	0	isocratic

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, R , between paclitaxel and benzyl analog is not less than 1.8. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%. [NOTE—For the purpose of peak identification, the approximate relative retention times are given in *Table 3*. The relative retention times are measured versus Paclitaxel.]

Table 3

Name	Relative Retention Time	Limit (%)
Propyl analog ¹	0.54	0.2
Cephalomannine (Paclitaxel related compound A)	0.76	0.5
sec-Butyl analog ²	0.81	0.2
n-Butyl analog ³	0.89	0.1
Benzyl analog	1.10	0.4
Baccatin VI	1.23	0.2
Pentyl analog ⁴	1.31	0.2
7-Epipaclitaxel	1.51	0.4

¹ The following chemical name is assigned to the related compound Propyl analog: Baccatin III 13-ester with (2*R*,3*S*)-3-butanoylamino-2-hydroxy-3-phenylpropanoic acid.

² The following chemical name is assigned to the related compound sec-Butyl analog: Baccatin III 13-ester with (2*R*,3*S*)-2-hydroxy-3-(2-methylbutanoylamino)-3-phenylpropanoic acid.

³ The following chemical name is assigned to the related compound n-Butyl analog: Baccatin III 13-ester with (2*R*,3*S*)-2-hydroxy-3-(pentanoylamino)-3-phenylpropanoic acid.

⁴ The following chemical name is assigned to the related compound Pentyl analog: Baccatin III 13-ester with (2*R*,3*S*)-3-(hexanoylamino)-2-hydroxy-3-phenylpropanoic acid.

Procedure—Inject a volume (about 12 μL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for all the peaks. Calculate the per-

centage of each impurity in the portion of Paclitaxel taken by the formula:

$$100(r_i / r_U)$$

in which r_i is the response of each individual impurity; and r_U is the sum of the areas of all the peaks obtained from the *Test solution*. In addition to not exceeding the limits for paclitaxel related impurities in *Table 3*, not more than 0.1% of any other single impurity is found; and not more than 2.0% of total impurities is found.

Assay—

Diluent—Prepare a mixture of methanol and acetic acid (200:1).

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (11:9). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

Standard preparation—Dissolve, using sonication if necessary, an accurately weighed quantity of USP Paclitaxel RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 1 mg per mL.

Assay preparation—Transfer about 10 mg of Paclitaxel, accurately weighed, to a 10-mL volumetric flask. Dissolve in *Diluent*, using sonication if necessary, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L43. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is between 0.7 and 1.3; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{47}H_{51}NO_{14}$ in the portion of Paclitaxel taken by the formula:

$$10C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Paclitaxel RS in the *Standard preparation*; and r_U and r_S are the peak responses for paclitaxel obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Paclitaxel Injection

» Paclitaxel Injection is a sterile, stabilized solution of Paclitaxel, suitable for dilution for intravenous administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of paclitaxel ($C_{47}H_{51}NO_{14}$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, at controlled room temperature.

Labeling—Label it to indicate that it is to be diluted with a suitable parenteral vehicle prior to intravenous infusion.

USP Reference standards (11)—

USP Endotoxin RS

USP Paclitaxel RS

USP Paclitaxel Related Compound B RS

10-Deacetyl-7-epipaclitaxel.

Identification—

A: The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the test for *Limit of degradation products*.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test (85)—It contains not more than 0.67 USP Endotoxin Unit per mg of paclitaxel.

pH (791): between 3.0 and 7.0, in a solution (1 in 10).

Limit of degradation products—

Solution A—Prepare a filtered and degassed mixture of water and acetonitrile (3:2).

Solution B—Use filtered and degassed acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard solution—Dissolve accurately weighed quantities of USP Paclitaxel RS and USP Paclitaxel Related Compound B RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, to obtain solutions having known concentrations of about 1.2 mg per mL and 0.006 mg per mL, respectively.

Test solution—Quantitatively dilute an accurately measured volume of *Injection* with acetonitrile to obtain a solution containing about 1.2 mg of paclitaxel per mL, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 227-nm detector and a 4.6-mm × 15-cm column that contains 3-μm packing L1. The flow rate is about 1.2 mL per minute. The column temperature is maintained at 35°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–26	100	0	isocratic
26–66	100→17	0→83	linear gradient
66–67	17→100	83→0	linear gradient
67–75	100	0	isocratic

Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution, R , between paclitaxel related compound B and paclitaxel is not less than 1.2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the analyte peaks. Calculate the percentage of each degradation product in the volume of *Injection* taken by the formula:

$$100(C_S / C_U)(r_i / r_S)$$

in which C_S is the concentration, in mg per mL, of USP Paclitaxel Related Compound B RS in the *Standard solution*; C_U is the concentration, in mg per mL, of paclitaxel in the *Test solution*, based on the labeled amount of paclitaxel per mL of *Injection*; r_i is the peak area for each degradation product obtained from the *Test solution*; and r_S is the peak area for paclitaxel related compound B obtained from the *Standard solution*. In addition to not exceeding the limits stated in *Table 1*, not more than 0.1% of any other paclitaxel degradation product is found; and not more than 2.0% of total paclitaxel degradation products is found.

Table 1

Relative Retention Time	Name	Limit (%)
0.19	Baccatin III	0.8
0.21	Ethyl ester side chain	0.4
0.50	10-Deacetylpaclitaxel	0.8
0.95	10-Deacetyl-7-epipaclitaxel (paclitaxel related compound B)	0.5
1.40	7-Epipaclitaxel	0.6

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Diluent—Transfer 200 μL of glacial acetic acid to a 1-liter volumetric flask containing about 500 mL of methanol, mix, and dilute with methanol to volume.

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (11:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Paclitaxel RS in *Diluent* to obtain a solution having a known concentration of about 0.6 mg per mL.

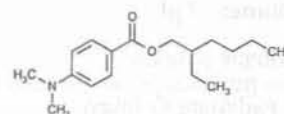
Assay preparation—Quantitatively dilute an accurately measured volume of *Injection* with *Diluent* to obtain a solution containing about 0.6 mg of paclitaxel per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 227-nm detector and a 4.0-mm × 25-cm column that contains 5-μm packing L43. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the retention time of the paclitaxel peak is between 6.0 and 10.0 minutes; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the paclitaxel peaks. Calculate the quantity, in mg, of paclitaxel ($C_{47}H_{51}NO_{14}$) in each mL of the *Injection* taken by the formula:

$$(L/D)C(r_U / r_S)$$

in which L is the labeled quantity, in mg, of paclitaxel in each mL of *Injection*; D is the concentration, in mg per mL, of paclitaxel in the *Assay preparation*, based on the labeled quantity; C is the concentration, in mg per mL, of USP Paclitaxel RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Padimate O

$C_{17}H_{27}NO_2$ 277.40
Benzoic acid, 4-(dimethylamino)-, 2-ethylhexyl ester;
2-Ethylhexyl *p*-(dimethylamino)benzoate [21245-02-3].

DEFINITION

Padimate O contains NLT 97.0% and NMT 102.0% of padimate O ($C_{17}H_{27}NO_2$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197F)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Mobile phase: Methanol, water, and glacial acetic acid (850:150:0.5)

Standard solution: 0.1 mg/mL of USP Padimate O RS in methanol

Sample solution: 0.1 mg/mL of Padimate O in methanol

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 308 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of padimate O (C₁₇H₂₇NO₂) in the portion of Padimate O taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Padimate O RS in the *Standard solution* (mg/mL)

C_u = concentration of Padimate O in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0%

IMPURITIES• **ORGANIC IMPURITIES**

Sample solution: 10 mg/mL of Padimate O in chloroform

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 3-mm × 1.8-m stainless steel packed with 10% liquid phase G9 on support S1A

Carrier gas: Helium

Column temperature: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
150	10	250	10

Injection volume: 2 μL

Analysis

Sample: *Sample solution*

Calculate the percentage of the total impurities in the portion of Padimate O taken:

$$[\Sigma r_i / (\Sigma r_i + r_u)] \times 100$$

r_i = individual impurity peak response from the *Sample solution*

r_u = Padimate O peak response from the *Sample solution*

Acceptance criteria

Total impurities: NMT 2.0%

SPECIFIC TESTS

• **SPECIFIC GRAVITY** (841): 0.990–1.000

• **REFRACTIVE INDEX** (831): 1.5390–1.5430

• **FATS AND FIXED OILS, Acid Value** (401): NMT 1.0

• **FATS AND FIXED OILS, Saponification Value** (401)

Analysis: Proceed as directed in the chapter, except to maintain reflux for 4 h.

Acceptance criteria: 195–215

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** (11)

USP Padimate O RS

Padimate O Lotion

» Padimate O Lotion contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C₁₇H₂₇NO₂.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Padimate O RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Assay—

Mobile phase—Prepare a suitable filtered and degassed solution containing methanol, water, and glacial acetic acid (85:15:0.5).

Standard preparation—Dissolve an accurately weighed quantity of USP Padimate O RS in isopropyl alcohol and dilute quantitatively, and stepwise if necessary, with isopropyl alcohol to obtain a solution having a known concentration of about 100 μg per mL.

Assay preparation—Transfer an accurately weighed quantity of Lotion, equivalent to about 100 mg of Padimate O, to a 100-mL volumetric flask, and add about 75 mL of isopropyl alcohol. Heat gently with swirling until the specimen is dispersed. Cool to room temperature, dilute with isopropyl alcohol to volume, and mix. Pipet 10.0 mL of this solution into a 100-mL volumetric flask, dilute with isopropyl alcohol to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 308-nm detector and a 4.6-mm × 25-cm column that contains 5-μm base-deactivated packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*; the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C₁₇H₂₇NO₂ in the portion of Lotion taken by the formula:

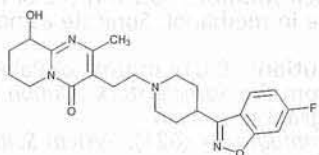
$$C(r_u / r_s)$$

in which C is the concentration, in μg per mL, of USP Padimate O RS in the *Standard preparation*, and r_u and r_s are the

peak responses for padimate O obtained from the Assay preparation and the Standard preparation, respectively.

Paliperidone

Change to read:



$C_{23}H_{27}FN_4O_3$ 426.48
4*H*-Pyrido[1,2-*a*]pyrimidin-4-one, 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-;
• (9*RS*)-3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]]ethyl-9-hydroxy-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one • (ERR 1-Jun-2016) [144598-75-4].

DEFINITION

Paliperidone contains NLT 98.0% and NMT 102.0% of paliperidone ($C_{23}H_{27}FN_4O_3$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K): Do not dry the sample.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Protect the *System suitability solution*, *Standard solution*, and *Sample solution* from light.

Buffer: 28 mM Tetrabutylammonium hydrogen sulfate

Mobile phase: See Table 1.

Solution A: Methanol and Buffer (10:90)

Solution B: Methanol

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
35	85	15
37	100	0
45	100	0

Diluent: Dissolve 0.71 g of dibasic sodium phosphate and 0.62 g of monobasic sodium phosphate in 1 L of water.

System suitability solution: 0.5 mg/mL of USP Paliperidone Resolution Mixture RS prepared as follows. Dissolve first in methanol using 50% of the final flask volume, and dilute with *Diluent*.

Standard solution: 0.5 mg/mL of USP Paliperidone RS prepared as follows. Dissolve first in methanol using 50% of the final flask volume, and dilute with *Diluent*.

Sample solution: 0.5 mg/mL of Paliperidone prepared as follows. Dissolve first in methanol using 50% of the final flask volume, and dilute with *Diluent*.

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 10-cm; 3-μm packing L1

Column temperature: 40°

Flow rate: 0.9 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between paliperidone related compound B and paliperidone; NLT 2.0 between paliperidone and paliperidone hydroxybenzoyl analog, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of paliperidone ($C_{23}H_{27}FN_4O_3$) in the portion of Paliperidone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Paliperidone RS in the *Standard solution* (mg/mL)

C_U = concentration of Paliperidone in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Change to read:

• ORGANIC IMPURITIES

Protect the *System suitability solution*, *Standard solution*, and *Sample solution* from light.

Diluent, Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Sensitivity solution: 0.5 μg/mL of USP Paliperidone RS in *Diluent* from *Standard solution*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—See Table 2 for relative retention times.]

Suitability requirements

Resolution: NLT 2.0 between paliperidone related compound B and paliperidone; NLT 2.0 between paliperidone and paliperidone hydroxybenzoyl analog, *System suitability solution* (ERR 1-Jun-2016)

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Relative standard deviation: NMT 5.0%, *Sensitivity solution*

Analysis:

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Paliperidone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response of paliperidone from the *Standard solution*

C_S = concentration of USP Paliperidone RS in the *Standard solution* (mg/mL)

C_U = concentration of Paliperidone in the *Sample solution* (mg/mL)

F = relative response factor for the corresponding impurity (see Table 2)

Acceptance criteria: See Table 2. Disregard any peak with area less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Paliperidone related compound C ^a	0.57	1.0	0.10
Paliperidone related compound B ^b	0.83	1.0	0.10
Paliperidone	1.00	—	—
Paliperidone hydroxybenzoyl analog ^c	1.1	1.0	0.10
Paliperidone ketone ^d	1.27	0.58	0.50
Any other individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.70

^a 3-(2-Chloroethyl)-9-hydroxy-2-methyl-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one.

^b 6-Fluoro-3-(piperidin-4-yl)benzoxazole.

^c 3-[2-[4-(4-Fluoro-2-hydroxybenzoyl)piperidin-1-yl]ethyl]-9-hydroxy-2-methyl-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one.

^d 3-[2-[4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-7,8-dihydro-4H-pyrido[1,2-a]pyrimidine-4,9(6H)-dione.

SPECIFIC TESTS

- **WATER DETERMINATION, Method 1a (921):** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

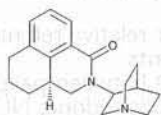
- **USP REFERENCE STANDARDS (11)**

USP Paliperidone RS

USP Paliperidone Resolution Mixture RS

This contains paliperidone as the major component; it also contains paliperidone related compound B, paliperidone related compound C, paliperidone hydroxybenzoyl analog, and paliperidone ketone as minor components.

Palonosetron Hydrochloride



$C_{19}H_{24}N_2O \cdot HCl$ 332.87
1H-Benzo[de]isoquinoline-1-one, 2,3,3a,4,5,6-hexahydro-2-[(3S)-1-azabicyclo[2.2.2]octan-3-yl]-5,6-dihydro-1H-benzod[e]isoquinolin-1-one hydrochloride; (3aS)-2-[(3S)-Quinuclidin-3-yl]-2,3,3a,4,5,6-hexahydro-1H-benzo[de]isoquinolin-1-one hydrochloride [135729-62-3].

DEFINITION

Palonosetron Hydrochloride contains NLT 98.0% and NMT 102.0% of palonosetron hydrochloride ($C_{19}H_{24}N_2O \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (17K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile, water, and trifluoroacetic acid (280: 720: 0.67)

Standard stock solution: 0.7 mg/mL of USP Palonosetron Hydrochloride RS in methanol. Sonicate as needed to dissolve. This solution is stable for 6 weeks if stored in the refrigerator and protected from light.

Standard solution: 0.014 mg/mL of USP Palonosetron Hydrochloride RS from the *Standard stock solution* in *Mobile phase*

Sample stock solution: 0.7 mg/mL of Palonosetron Hydrochloride in methanol. Sonicate as needed to dissolve.

Sample solution: 0.014 mg/mL of Palonosetron Hydrochloride from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Flow rate: 1 mL/min

Injection volume: 80 μL

System suitability

Sample: *Standard solution*

[NOTE—See Table 2 for the relative retention times.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of palonosetron hydrochloride ($C_{19}H_{24}N_2O \cdot HCl$) in the portion of Palonosetron Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Palonosetron Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Palonosetron Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

- **LIMIT OF SPECIFIED IMPURITIES**

Buffer: 1.5 g/L of ammonium acetate, adjusted with glacial acetic acid to a pH of 6.0

Mobile phase: Tetrahydrofuran and *Buffer* (1:9)

System suitability stock solution: 0.07 mg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound C RS, USP Palonosetron Related Compound D RS, USP Palonosetron Related Compound E RS, and USP Palonosetron Enantiomer RS in methanol. Sonicate as needed to dissolve.

System suitability solution: 3.5 μg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound C RS, USP Palonosetron Related Compound D RS, USP Palonosetron Related Compound E RS, and USP Palonosetron Enantiomer RS from the *System suitability stock solution* and 0.7 mg/mL of USP Palonosetron Hydrochloride RS in *Buffer*

Impurity standard stock solution: 0.35 mg/mL of USP Palonosetron Related Compound D RS in methanol. Sonicate as needed to dissolve.

Impurity standard solution: 0.0035 mg/mL of USP Palonosetron Related Compound D RS from the *Impurity standard stock solution* in *Buffer*

Sensitivity solution: 0.35 µg/mL of USP Palonosetron Related Compound D RS from the *Impurity standard stock solution* in *Buffer*

Sample solution: 0.7 mg/mL of Palonosetron Hydrochloride in *Buffer*. Sonicate as needed to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 238 nm

Column: 4.6-mm × 25-cm; 5-µm packing L88

Column temperature: 35°

Flow rate: 1.5 mL/min

Injection volume: 100 µL

Run time: At least 2.5 times the retention time of palonosetron

System suitability

Samples: *System suitability solution*, *Impurity standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.4 between palonosetron related compound D and palonosetron enantiomer; NLT 1.2 between palonosetron enantiomer and palonosetron related compound E, *System suitability solution*

Tailing factor: NMT 2.5 for palonosetron related compound E, *System suitability solution*

Relative standard deviation: NMT 5.0% for palonosetron related compound D, *Impurity standard solution*

Signal-to-noise ratio: NLT 10 for palonosetron related compound D, *Sensitivity solution*

Analysis

Samples: *Impurity standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Palonosetron Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of palonosetron related compound D from the *Impurity standard solution*

C_S = concentration of USP Palonosetron Related Compound D RS in the *Impurity standard solution* (mg/mL)

C_U = concentration of Palonosetron Hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Palonosetron related compound A	0.45	1.0	0.1
Palonosetron related compound B	0.54	2.1	0.1
Palonosetron	1.0	—	—
Palonosetron related compound C	1.2	1.0	0.1
Palonosetron related compound D	1.3	1.0	0.5
Palonosetron enantiomer	1.4	1.0	0.1
Palonosetron related compound E	1.5	2.5	0.5

• LIMIT OF UNSPECIFIED IMPURITIES

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*.

Peak identification stock solution: 0.07 mg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound D RS, and USP Palonosetron Related Compound E RS in methanol. Sonicate as needed to dissolve.

Peak identification solution: 0.07 µg/mL each of USP Palonosetron Related Compound A RS, USP Palonosetron Related Compound B RS, USP Palonosetron Related Compound D RS, and USP Palonosetron Related Compound E RS from the *Peak identification stock solution* and 3.5 µg/mL of USP Palonosetron Hydrochloride RS from the *Standard stock solution* in *Mobile phase*

Sensitivity solution: 0.0175 µg/mL of USP Palonosetron Hydrochloride RS in *Mobile phase*

Sample stock solution: 0.175 mg/mL of Palonosetron Hydrochloride in methanol. Sonicate as needed to dissolve.

Sample solution: 0.035 mg/mL of Palonosetron Hydrochloride from the *Sample stock solution* in *Mobile phase*

System suitability

Sample: *Sensitivity solution*

Suitability requirements

Signal-to-noise ratio: NLT 10

Analysis

Samples: *Peak identification solution* and *Sample solution*

Chromatograph the *Peak identification solution* and identify specified impurities based on the relative retention times listed in *Table 2*.

Calculate the percentage of each unspecified impurity in the portion of Palonosetron Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each unspecified impurity from the *Sample solution*

r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Palonosetron related compound E ^a	0.91	—
Palonosetron related compound D ^a	0.94	—
Palonosetron	1.0	—
Palonosetron related compound B ^a	1.1	—
Palonosetron related compound A ^a	1.2	—

^aThese impurities are controlled in the test for *Limit of Specified Impurities* and are not to be reported here.

^bTotal impurities include impurities controlled in the test for *Limit of Specified Impurities* and in the test for *Limit of Unspecified Impurities*.

Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	0.1
Total impurities ^b	—	1.0

^a These impurities are controlled in the test for *Limit of Specified Impurities* and are not to be reported here.

^b Total impurities include impurities controlled in the test for *Limit of Specified Impurities* and in the test for *Limit of Unspecified Impurities*.

SPECIFIC TESTS

• pH (791)

Sample solution: 10 mg/mL in water
Acceptance criteria: 5.0–6.0

• LOSS ON DRYING (731)

Analysis: Dry under vacuum at 80° for 3 h.
Acceptance criteria: NMT 1.0%

• MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):

The total aerobic microbial count does not exceed 10² cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g.

• BACTERIAL ENDOTOXINS TEST (85):

Where the label states that Palonosetron Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 30 USP Endotoxin Units/mg of palonosetron hydrochloride.

• STERILITY TESTS (71):

Meets the requirements where the label states that Palonosetron Hydrochloride is sterile

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in well-closed containers. Store at controlled room temperature, protected from light.

• LABELING:

Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• USP REFERENCE STANDARDS (11)

USP Endotoxin RS
USP Palonosetron Enantiomer RS
(3*aR*)-2-[(3*R*)-Quinuclidin-3-yl]-2,3,3*a*,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride.

C₁₉H₂₄N₂O · HCl 332.87

USP Palonosetron Hydrochloride RS

USP Palonosetron Related Compound A RS

Palonosetron *N*-oxide;
(3*aS*)-1-Oxo-2-[(3*S*)-quinuclidin-3-yl]-2,3,3*a*,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinoline 1-oxide.

C₁₉H₂₄N₂O₂ 312.41

USP Palonosetron Related Compound B RS

Palonosetron-3-ene *N*-oxide;
(3*S*)-3-(1-Oxo-5,6-dihydro-1*H*-benzo[*de*]isoquinolin-2(4*H*)-yl)quinuclidine 1-oxide.

C₁₉H₂₂N₂O₂ 310.39

USP Palonosetron Related Compound C RS

Palonosetron *S*,*R*-diastereomer;
(3*aS*)-2-[(3*R*)-Quinuclidin-3-yl]-2,3,3*a*,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride.

C₁₉H₂₄N₂O · HCl 332.87

USP Palonosetron Related Compound D RS

Palonosetron *R*,*S*-diastereomer or palonosetron 3*a*-epimer;
(3*aR*)-2-[(3*S*)-Quinuclidin-3-yl]-2,3,3*a*,4,5,6-hexahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride.

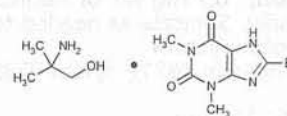
C₁₉H₂₄N₂O · HCl 332.87

USP Palonosetron Related Compound E RS

Palonosetron-3-ene;
2-[(3*S*)-Quinuclidin-3-yl]-2,4,5,6-tetrahydro-1*H*-benzo[*de*]isoquinolin-1-one hydrochloride.

C₁₉H₂₂N₂O · HCl 330.85

Pamabrom



C₁₁H₁₈BrN₅O₃ 348.20

8-Bromo-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione compound with 2-amino-2-methyl-1-propanol (1:1).

8-Bromotheophylline compound with 2-amino-2-methyl-1-propanol (1:1) [606-04-02].

» Pamabrom contains not less than 72.2 percent and not more than 76.6 percent of 8-bromotheophylline (C₇H₇BrN₄O₂), calculated on the anhydrous basis; and not less than 24.6 percent and not more than 26.6 percent of 2-amino-2-methyl-1-propanol (C₄H₁₁NO), calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP 8-Bromotheophylline RS
8-Bromo-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione.
C₇H₇N₄O₂Br 259.06

USP Theophylline RS

Identification—It responds to the *Thin-layer Chromatographic Identification Test* (201), using a solvent system consisting of a mixture of xylene, methanol, and glacial acetic acid (11:2:1) and a *Standard solution* and a *Test solution* prepared as directed below: the *R_f* value of the principal spot, which appears as a dark spot against a green background, from the *Test solution* corresponds to that obtained from the *Standard solution*.

Standard solution—Transfer an accurately weighed quantity of about 20 mg of USP 8-Bromotheophylline RS to a 100-mL volumetric flask, add 25 mL of water, 50 mL of methanol, and a small amount of dilute ammonium hydroxide. Swirl the flask to effect solution. Dilute the contents of the flask with methanol to volume, and mix.

Test solution—Transfer an accurately weighed quantity of about 25 mg of Pamabrom to a 100-mL volumetric flask, add 25 mL of water, and swirl to dissolve. Dilute the contents of the flask with methanol to volume, and mix.

Water Determination, Method I (921): not more than 3%.

Delete the following:

• **Heavy metals, Method II** (231): 20 µg per g. • (Official 1-Jan-2018)

Limit of theophylline—

Diluting solution, Mobile phase, and Chromatographic system—Proceed as directed in the *Assay for 8-bromotheophylline*.

Standard solution—Dissolve an accurately weighed quantity of USP Theophylline RS in *Diluting solution*, add a few drops of ammonium hydroxide, sonicate if necessary, to obtain a solution having a known concentration of about 1 mg of USP Theophylline RS per mL. Dilute a volume of this solution quantitatively, and stepwise if necessary, with *Diluting solution* to obtain a solution having a known concentration of about 5 µg per mL.

Test solution—Transfer an accurately weighed quantity of about 200 mg of Pamabrom to a 200-mL volumetric flask. Add about 50 mL of *Diluting solution*, and sonicate for

5 minutes. Cool to room temperature, dilute with *Diluting solution* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of theophylline in the portion of Pamabrom taken by the formula:

$$20(C/W)(r_U/r_S)$$

in which C is the concentration, in μ g per mL, of USP Theophylline RS in the *Standard solution*, W is the weight, in mg, of Pamabrom taken, and r_U and r_S are the peak responses of theophylline obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.5% is found.

Assay for 8-bromotheophylline—

Diluting solution—Prepare a mixture of water and methanol (70:30).

Mobile phase—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (69:30:1), filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard solution—Dissolve an accurately weighed quantity of caffeine in *Diluting solution*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a concentration of about 125 μ g of caffeine per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP 8-Bromotheophylline RS in *Diluting solution*, add a few drops of ammonium hydroxide, sonicating if necessary, to obtain a solution having a known concentration of about 0.75 mg of USP 8-Bromotheophylline RS per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, mix, and filter.

Assay preparation—Transfer an accurately weighed quantity of about 200 mg of Pamabrom to a 200-mL volumetric flask, add about 50 mL of *Diluting solution* and two drops of ammonium hydroxide, and sonicate for 5 minutes. [NOTE—If a hazy solution is present after 5 minutes of sonication, add 1 additional drop of ammonium hydroxide.] Cool, dilute with *Diluting solution* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, mix, and filter.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm \times 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph 20 μ L of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.6 for caffeine and 1.0 for 8-bromotheophylline, the resolution, R , between caffeine and 8-bromotheophylline is not less than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of 8-bromotheophylline ($C_7H_7BrN_4O_2$) in the portion of Pamabrom taken by the formula:

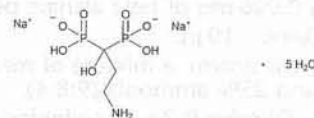
$$4000C(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP 8-Bromotheophylline RS in the *Standard preparation*, and R_U and R_S are the peak response ratios of the 8-bromotheophylline peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for 2-amino-2-methyl-1-propanol—Dissolve about 1 g of Pamabrom, accurately weighed, in 10 mL of water by warming gently on a steam bath until the solution

is clear. Cool, add methyl orange TS, and titrate with 0.5 N hydrochloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.5 N hydrochloric acid is equivalent to 44.57 mg of $C_4H_{11}NO$.

Pamidronate Disodium



$C_3H_9NNa_2O_7P_2 \cdot 5H_2O$ 369.11

Phosphonic acid, (3-amino-1-hydroxypropylidene)bis-, disodium salt, pentahydrate.

Disodium dihydrogen (3-amino-1-hydroxypropylidene)diphosphonate, pentahydrate [109552-15-0].

Anhydrous 279.06 [57248-88-1].

» Pamidronate Disodium contains not less than 98.0 percent and not more than 102.0 percent of $C_3H_9NNa_2O_7P_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers. Store at a temperature not exceeding 30°.

USP Reference standards (11)—

USP Beta Alanine RS

3-Aminopropionic acid.

USP Pamidronate Disodium RS

Clarity and color of solution—

Test preparation 1—Dissolve 1.0 g in 50 mL of water with gentle warming. Cool to room temperature.

Test preparation 2—Dissolve 1.0 g in 25 mL of 2 N sodium hydroxide solution with gentle warming. Cool to room temperature.

Procedure—Examine *Test preparation 1* and *Test preparation 2*: the solutions are clear. Separately measure the absorbance of each of these solutions at 420 nm in 4-cm cells, using water as the blank for *Test preparation 1* and using 2 N sodium hydroxide solution as the blank for *Test preparation 2*: the absorbance of each solution is not more than 0.10.

Identification—

A: Infrared Absorption (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

C: It meets the requirements of the pyroantimonate precipitate test for Sodium (191).

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—The total aerobic microbial count does not exceed 1000 cfu per g, and the total combined yeasts and molds count does not exceed 100 cfu per g.

pH (791): between 7.8 and 8.8, in a solution (1 in 100).

Water Determination, Method I (921): between 23.0% and 25.5%.

Delete the following:

• **Heavy metals, Method II** (231): 20 μ g per g. • (Official 1-Jan-2018)

Related compounds—**TEST 1—**

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Test solution—Transfer 30 mg of Pamidronate Disodium to a 10-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Standard solution—Dissolve an accurately weighed quantity of USP Beta Alanine RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution containing 0.006 mg of beta alanine per mL.

Application volume: 10 μ L.

Developing solvent system: a mixture of methanol, diisopropyl ether, and 25% ammonia (9:8:4).

Spray reagent—Dissolve 0.2 g of ninhydrin in 100 mL of a mixture of butyl alcohol and 2 N acetic acid (95:5).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Dry the plate between 100° and 105° until the ammonia disappears completely. Spray with *Spray reagent*, and heat between 100° and 105° for about 15 minutes. Examine the plate under white light. The spot obtained from the *Test solution* having an R_f value of about 0.5 is not greater in size or intensity than the corresponding spot obtained from the *Standard solution*: not more than 0.2% of beta alanine is found. Evaluate any other additional spot in the chromatogram of the *Test solution*, and determine the percentage of total other impurities (excluding beta alanine).

TEST 2—

Mobile phase—Proceed as directed in the *Assay*.

Impurity stock solution 1—Transfer about 300 mg of ortho-phosphoric acid, accurately weighed, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Impurity stock solution 2—Transfer about 250 mg of phosphorous acid, accurately weighed, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Impurity standard solution—Transfer 2.0 mL each of *Impurity stock solution 1* and *Impurity stock solution 2* to a 50-mL volumetric flask, dilute with water to volume, and mix.

Test solution—Prepare as directed for the *Assay preparation*.

Chromatographic system (see *Chromatography* (621))—Proceed as directed in the *Assay*. Chromatograph the *Impurity standard solution*, and record the peak responses as directed for *Procedure*: the elution order is a phosphate peak followed by the phosphite peak; the resolution, R , between the two peaks is not less than 2.5; the relative standard deviation for replicate injections, determined from the phosphate peak, is not more than 10%; and the relative standard deviation for replicate injections, determined from the phosphite peak, is not more than 20%.

Procedure—Separately inject equal volumes (about 100 μ L) of the *Impurity standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of phosphates as ortho-phosphoric acid in the portion of Pamidronate Disodium taken by the formula:

$$0.2(W_1 / W)(r_u / r_s)$$

in which W_1 is the weight, in mg, of ortho-phosphoric acid taken to prepare the *Impurity stock solution 1*; W is the weight, in mg, of Pamidronate Disodium taken to prepare the *Test solution*; and r_u and r_s are the phosphate peak responses obtained from the *Test solution* and the *Impurity standard solution*, respectively: not more than 0.5% of phosphate, determined as ortho-phosphoric acid, is found.

Calculate the percentages of phosphites as phosphorous acid in the portion of Pamidronate Disodium taken by the formula:

$$0.2(W_2 / W)(r_u / r_s)$$

in which W_2 is the weight, in mg, of phosphorous acid taken to prepare the *Impurity stock solution 2*; W is as defined above; and r_u and r_s are the phosphite peak responses obtained from the *Test solution* and the *Impurity standard solution*, respectively: not more than 0.5% of phosphite, determined as phosphorous acid, is found; and not more than 0.5% of total phosphate and phosphite combined is found.

Calculate the percentage of any other impurity in the portion of Pamidronate Disodium taken by the formula:

$$0.2(W_1 / W)(r_i / r_s)$$

in which W_1 and W are as defined above; r_i is the peak response of any other impurity in the *Test solution*; and r_s is the response of the phosphate peak obtained from the *Impurity standard solution*: not more than 0.5% of total other impurities (excluding beta alanine, phosphate as ortho-phosphoric acid, and phosphite as phosphorous acid) is found, the results for *Test 1* and *Test 2* being added.

Assay—

Mobile phase—To 2500 mL of water, add 0.47 mL of anhydrous formic acid, adjust with 2 N sodium hydroxide solution to a pH of 3.5, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—The small amounts of formic acid have a strong influence on the retention times.]

Standard preparation—Dissolve an accurately weighed quantity of USP Pamidronate Disodium RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 2 mg per mL.

Assay preparation—Transfer about 100 mg of Pamidronate Disodium, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a refractive index detector and a 4.6-mm \times 10-cm column that contains packing L23. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 35°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not less than 0.3 and not more than 1.2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 100 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_3H_5NNa_2O_7P_2$ in the portion of Pamidronate Disodium taken by the formula:

$$50C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Pamidronate Disodium RS in the *Standard preparation*; and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pamidronate Disodium for Injection

» Pamidronate Disodium for Injection is a sterile, freeze-dried mixture of Pamidronate Disodium and suitable excipients. It contains not less than 93.0 percent and not more than 108.0 percent of

the labeled amount of pamidronate disodium ($\text{C}_3\text{H}_9\text{NNa}_2\text{O}_7\text{P}_2$).

Change to read:

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017). Store at controlled room temperature.

USP Reference standards (11)—

USP Beta Alanine RS

3-Aminopropionic acid.

USP Endotoxin RS

USP Pamidronate Disodium RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test (85)—It contains not more than 3.88 USP Endotoxin Units per mg of anhydrous pamidronate disodium.

Uniformity of dosage units (905): meets the requirements for *Weight Variation*.

pH (791): between 6.0 and 7.0, determined in a solution constituted as directed in the labeling.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Water Determination, Method 1a (921): not more than 5%.

Limit of beta alanine—

Adsorbent, Application volume, Developing solvent system, and Spray reagent—Proceed as directed for *Related compounds, Test T* under *Pamidronate Disodium*.

Standard solution—Dissolve an accurately weighed quantity of USP Beta Alanine RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution containing 0.0075 mg of beta alanine per mL.

Test solution—Reconstitute the vial with the appropriate amount of water to obtain a solution having a concentration of 3 mg of anhydrous pamidronate disodium per mL, based on the label claim.

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Dry the plate between 100° and 105° until the ammonia disappears completely. Spray with *Spray reagent*, and heat between 100° and 105° for about 15 minutes. Examine the plate under white light. The spot having an R_f value of about 0.5 obtained from the *Test solution* is not greater in size or intensity than the corresponding spot obtained from the *Standard solution*: not more than 0.25% of beta alanine is found.

Other requirements—It meets the requirements under *Sterility Tests* (71) and for *Labeling* (7), *Labels and Labeling for Injectable Products*.

Assay—

Mobile phase and Chromatographic system—Proceed as directed in the *Assay* under *Pamidronate Disodium*.

Standard preparation—Dissolve an accurately weighed quantity of USP Pamidronate Disodium RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 2.5 mg per mL. Calculate the concentration, C_s , of anhydrous pamidronate disodium, the molecular weights of anhydrous and pentahydrate pamidronate disodium being 279.06 and 369.11, respectively.

Assay preparation—Constitute a suitable number of vials of Pamidronate Disodium for Injection with the appropriate

amount of water to obtain a solution having a known concentration of about 2 mg of anhydrous pamidronate disodium per mL, based on the label claim.

Procedure—Separately inject equal volumes (about 100 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $\text{C}_3\text{H}_9\text{NNa}_2\text{O}_7\text{P}_2$ in the portion of Pamidronate Disodium for Injection taken by the formula:

$$100(C_s / C_u)(r_u / r_s)$$

in which C_s is as defined under the *Standard preparation*; C_u is the concentration, in mg per mL, of anhydrous pamidronate disodium in the *Assay preparation*; and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pancreatin

Pancreatin.

Pancreatin [8049-47-6].

» Pancreatin is a substance containing enzymes, principally amylase, lipase, and protease, obtained from the pancreas of the hog, *Sus scrofa* Linné var. *domesticus* Gray (Fam. Suidae) or of the ox, *Bos taurus* Linné (Fam. Bovidae). Pancreatin contains, in each mg, not less than 25 USP Units of amylase activity, not less than 2.0 USP Units of lipase activity, and not less than 25 USP Units of protease activity. Pancreatin of a higher digestive power may be labeled as a whole-number multiple of the three minimum activities or may be diluted by admixture with lactose, or with sucrose containing not more than 3.25 percent of starch, or with pancreatin of lower digestive power.

NOTE—One USP Unit of amylase activity is contained in the amount of pancreatin that decomposes starch at an initial rate such that 0.16 μEq of glycosidic linkage is hydrolyzed per minute under the conditions of the *Assay for amylase activity*.

One USP Unit of lipase activity is contained in the amount of pancreatin that liberates 1.0 μEq of acid per minute at a pH of 9.0 and 37° under the conditions of the *Assay for lipase activity*.

One USP Unit of protease activity is contained in the amount of pancreatin that under the conditions of the *Assay for protease activity* hydrolyzes casein at an initial rate such that there is liberated per minute an amount of peptides not precipitated by trichloroacetic acid that gives the same absorbance at 280 nm as 15 nmol of tyrosine.

Packaging and storage—Preserve in tight containers, at a temperature not exceeding 30°.

USP Reference standards (11)—

USP Bile Salts RS

USP Pancreatin Amylase and Protease RS

USP Pancreatin Lipase RS

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—It meets the requirements of the test for absence of *Salmonella* species and *Escherichia coli*.

Loss on drying (731)—Dry it in vacuum at 60° for 4 hours; it loses not more than 5.0% of its weight.

Fat—Place 2.0 g of Pancreatin in a flask of about 50-mL capacity, add 20 mL of ether, insert the stopper, and set it aside for 2 hours, mixing by rotating at frequent intervals. Decant the supernatant ether by means of a guiding rod into a plain filter about 7 cm in diameter, previously moistened with ether, and collect the filtrate in a tared beaker. Repeat the extraction with a 10-mL portion of ether, proceeding as directed before, then with another 10-mL portion of ether, and transfer the ether and the remainder of the Pancreatin to the filter. Allow to drain, evaporate the ether spontaneously, and dry the residue at 105° for 2 hours; the residue of fat obtained from Pancreatin possessing three or more times the three minimum activities weighs not more than 120 mg (6.0%); that obtained from Pancreatin possessing less than three times the three minimum activities weighs not more than 60 mg (3.0%).

Assay for amylase activity (Starch digestive power)—

pH 6.8 phosphate buffer—On the day of use, dissolve 13.6 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 14.2 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 51 mL of the monobasic potassium phosphate solution with 49 mL of the dibasic sodium phosphate solution. If necessary, adjust by the dropwise addition of the appropriate solution to a pH of 6.8.

Substrate solution—On the day of use, stir a portion of purified soluble starch equivalent to 2.0 g of dried substance with 10 mL of water, and add this mixture to 160 mL of boiling water. Rinse the beaker with 10 mL of water, add it to the hot solution, and heat to boiling, with continuous mixing. Cool to room temperature, and add water to make 200 mL.

Standard preparation—Weigh accurately about 20 mg of USP Pancreatin Amylase and Protease RS into a suitable mortar. Add about 30 mL of pH 6.8 phosphate buffer, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of pH 6.8 phosphate buffer to a 50-mL volumetric flask, dilute with pH 6.8 phosphate buffer to volume, and mix. Calculate the activity, in USP Units of amylase activity per mL, of the resulting solution from the declared potency on the label of the USP Reference Standard.

Assay preparation—For Pancreatin having about the same amylase activity as the USP Pancreatin Amylase and Protease RS, weigh accurately about 40 mg of Pancreatin into a suitable mortar. [NOTE—For Pancreatin having a different amylase activity, weigh accurately the amount necessary to obtain an Assay preparation having amylase activity per mL corresponding approximately to that of the Standard preparation.] Add about 3 mL of pH 6.8 phosphate buffer, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of pH 6.8 phosphate buffer to a 100-mL volumetric flask, dilute with pH 6.8 phosphate buffer to volume, and mix.

Procedure—Prepare four stoppered, 250-mL conical flasks, and mark them S, U, BS, and BU. Pipet into each flask 25 mL of Substrate solution, 10 mL of pH 6.8 phosphate buffer, and 1 mL of sodium chloride solution (11.7 in 1000), insert the stoppers, and mix. Place the flasks in a water bath maintained at 25 ± 0.1°, and allow them to equilibrate. To flasks BU and BS add 2 mL of 1 N hydrochloric acid, mix, and return the flasks to the water bath. To flasks U and BU add 1.0-mL portions of the Assay preparation, and to flasks S and BS add 1.0 mL of the Standard preparation. Mix each, and return the flasks to the water bath. After 10 minutes, accurately timed from the addition of the enzyme, add 2-mL portions of 1 N hydrochloric acid to flasks S and U, and mix. To each flask, with continuous stirring, add 10.0 mL of 0.1 N iodine VS, and immediately add 45 mL of

0.1 N sodium hydroxide. Place the flasks in the dark at a temperature between 15° and 25° for 15 minutes. To each flask add 4 mL of 2 N sulfuric acid, and titrate with 0.1 N sodium thiosulfate VS to the disappearance of the blue color. Calculate the amylase activity, in USP Units per mg, of the Pancreatin taken by the formula:

$$100(C_S / W_U)(V_{BU} - V_U) / (V_{BS} - V_S)$$

in which C_S is the amylase activity of the Standard preparation, in USP Units per mL, W_U is the amount, in mg, of Pancreatin taken, and V_U , V_S , V_{BU} , and V_{BS} are the volumes, in mL, of 0.1 N sodium thiosulfate consumed in the titration of the solutions in flasks U, S, BU, and BS, respectively.

Assay for lipase activity (Fat digestive power)—

Acacia solution—Centrifuge a solution of acacia (1 in 10) until clear. Use only the clear solution.

Olive oil substrate—Combine 165 mL of Acacia solution, 20 mL of olive oil, and 15 g of crushed ice in the cup of an electric blender. Cool the mixture in an ice bath to 5°, and homogenize at high speed for 15 minutes, intermittently cooling in an ice bath to prevent the temperature from exceeding 30°.

Test for suitability of mixing as follows. Place a drop of the homogenate on a microscope slide, and gently press a cover slide in place to spread the liquid. Examine the entire field under high power (43× objective lens and 5× ocular), using an eyepiece equipped with a calibrated micrometer. The substrate is satisfactory if 90% of the particles do not exceed 2 μm in diameter and none exceeds 10 μm in diameter.

Buffer solution—Dissolve 60 mg of tris(hydroxymethyl)aminomethane and 234 mg of sodium chloride in water to make 100 mL.

Bile salts solution—Prepare a solution to contain 80.0 mg of USP Bile Salts RS in each mL.

Standard test dilution—Suspend about 200 mg of USP Pancreatin Lipase RS, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 minutes, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based upon the declared potency on the label of the USP Reference Standard. Maintain the suspension at 4°, and mix before using. For each determination withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Assay test dilution—Suspend about 200 mg of Pancreatin, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 minutes, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based upon the estimated potency of the test material. Maintain the suspension at 4°, and mix before using. For each determination withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Procedure—Mix 10.0 mL of Olive oil substrate, 8.0 mL of Buffer solution, 2.0 mL of Bile salts solution, and 9.0 mL of water in a jacketed glass vessel of about 50-mL capacity, the outer chamber of which is connected to a thermostatically controlled water bath. Cover the mixture, and stir continuously with a mechanical stirring device. With the mixture maintained at a temperature of 37 ± 0.1°, add 0.1 N sodium hydroxide VS, from a microburet inserted through an opening in the cover, and adjust to a pH of 9.20 potentiometrically using a calomel-glass electrode system. Add 1.0 mL of the Assay test dilution, and then continue adding the 0.1 N sodium hydroxide VS for 5 minutes to maintain the pH at 9.0. Determine the volume of 0.1 N sodium hydroxide VS added after each minute.

In the same manner, titrate 1.0 mL of Standard test dilution.

Calculation of potency—Plot the volume of 0.1 N sodium hydroxide VS titrated against time. Using only the points

which fall on the straight-line segment of the curve, calculate the mean acidity released per minute by the test specimen and the Standard. Taking into consideration the dilution factors, calculate the lipase activity, in USP Units, of the Pancreatin taken by comparison to the activity of the Standard, using the lipase activity stated on the label of USP Pancreatin Lipase RS.

Assay for protease activity (Casein digestive power)—

Casein substrate—Place 1.25 g of finely powdered casein in a 100-mL conical flask containing 5 mL of water, shake to form a suspension, add 10 mL of 0.1 N sodium hydroxide, shake for 1 minute, add 50 mL of water, and shake for about 1 hour to dissolve the casein. The resulting solution should have a pH of about 8. If necessary, adjust the pH to about 8, using 1 N sodium hydroxide or 1 N hydrochloric acid. Transfer the solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Use this substrate on the day it is prepared.

Buffer solution—Dissolve 6.8 g of monobasic potassium phosphate and 1.8 g of sodium hydroxide in 950 mL of water in a 1000-mL volumetric flask, adjust to a pH of 7.5 ± 0.2 , using 0.2 N sodium hydroxide, dilute with water to volume, and mix. Store this solution in a refrigerator.

Trichloroacetic acid solution—Dissolve 50 g of trichloroacetic acid in 1000 mL of water. Store this solution at room temperature.

Filter paper—Determine the suitability of the filter paper by filtering a 5-mL portion of *Trichloroacetic acid solution* through the paper and measuring the absorbance of the filtrate at 280 nm, using an unfiltered portion of the same *Trichloroacetic acid solution* as the blank: the absorbance is not more than 0.04. If the absorbance is more than 0.04, the filter paper may be washed repeatedly with *Trichloroacetic acid solution* until the absorbance of the filtrate, determined as above, is not more than 0.04.

Standard test dilution—Add about 100 mg of USP Pancreatin Amylase and Protease RS, accurately weighed, to 100.0 mL of *Buffer solution*, and mix by shaking intermittently at room temperature for about 25 minutes. Dilute quantitatively with *Buffer solution* to obtain a concentration of about 2.5 USP Units of protease activity per mL, based on the potency declared on the label of the Reference Standard.

Assay test dilution—Weigh accurately about 100 mg of Pancreatin into a mortar. Add about 3 mL of *Buffer solution*, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *Buffer solution* to a 100-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Dilute quantitatively with *Buffer solution* to obtain a dilution that corresponds in activity to that of the *Standard test dilution*.

Procedure—Label test tubes in duplicate S_1 , S_2 , and S_3 for the standard series, and U for the sample. Pipet into tubes S_1 2.0 mL, into S_2 and U 1.5 mL, and into S_3 1.0 mL of *Buffer solution*. Pipet into tubes S_1 1.0 mL, into S_2 1.5 mL, and into S_3 2.0 mL of the *Standard test dilution*. Pipet into tubes U 1.5 mL of the *Assay test dilution*. Pipet into one tube each of S_1 , S_2 , S_3 , and U 5.0 mL of *Trichloroacetic acid solution*, and mix. Designate these tubes as S_{1B} , S_{2B} , S_{3B} , and U_B , respectively. Prepare a blank by mixing 3 mL of *Buffer solution* and 5 mL of *Trichloroacetic acid solution* in a separate test tube marked B . Place all the tubes in a 40° water bath, insert a glass stirring rod into each tube, and allow for temperature equilibration. At zero time, add to each tube, at timed intervals, 2.0 mL of the *Casein substrate*, preheated to the bath temperature, and mix. Sixty minutes, accurately timed, after the addition of the *Casein substrate* stop the reaction in tubes S_1 , S_2 , S_3 , and U by adding 5.0 mL of *Trichloroacetic acid solution* at the corresponding time intervals, stir, and remove all the tubes from the bath. Allow to stand for 10 minutes at room temperature for complete protein precipitation, and filter. The filtrates must be free from haze. Determine the absorbances of the filtrates, in 1-cm cells, at

280 nm, with a suitable spectrophotometer, using the filtrate from the blank (tube B) to set the instrument.

Calculation of potency—Correct the absorbance values for the filtrates from tubes S_1 , S_2 , and S_3 by subtracting the absorbance values for the filtrates from tubes S_{1B} , S_{2B} , and S_{3B} , respectively, and plot the corrected absorbance values against the corresponding volumes of the *Standard test dilution* used. From the curve, using the corrected absorbance value ($U - U_B$) for the Pancreatin taken, and taking into consideration the dilution factors, calculate the protease activity, in USP Units, of the Pancreatin taken by comparison with that of the Standard, using the protease activity stated on the label of USP Pancreatin Amylase and Protease RS.

Pancreatin Tablets

» Pancreatin Tablets contain not less than 90.0 percent of the labeled amount of pancreatin.

Packaging and storage—Preserve in tight containers, preferably at a temperature not exceeding 30°.

Labeling—Label the Tablets to indicate minimum pancreatic fat digestive power; i.e., single strength, double strength, triple strength.

USP Reference standards (11)—

USP Bile Salts RS

USP Pancreatin Amylase and Protease RS

USP Pancreatin Lipase RS

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—Tablets meet the requirements of the test for absence of *Salmonella* species and *Escherichia coli*.

Disintegration (701): 60 minutes.

Assay for amylase activity (Starch digestive power)—

Weigh and finely powder not fewer than 20 Tablets, avoiding the production of heat during the process. Proceed as directed in the *Assay for amylase activity* under *Pancreatin*, using as the assay preparation an accurately weighed portion of the powder, equivalent to 40 mg of pancreatin. (Use an inversely proportionate amount of the powder if the Tablets are labeled to contain a whole-number multiple of the minimum requirement for pancreatin digestive activity.)

Assay for lipase activity (Fat digestive power)—

Acacia solution, *Olive oil substrate*, *Buffer solution*, *Bile salts solution*, and *Standard test dilution*—Prepare as directed in the *Assay for lipase activity* under *Pancreatin*.

Assay test dilution—Proceed as directed for *Assay test dilution* in the *Assay for lipase activity* under *Pancreatin*, using as the assay preparation an accurately weighed portion of the powder, prepared as directed in the *Assay for amylase activity*, equivalent to about 200 mg of pancreatin.

Procedure and Calculation of potency—Proceed as directed in the *Assay for lipase activity* under *Pancreatin*.

Assay for protease activity (Casein digestive power)—

Casein substrate, *Buffer solution*, *Trichloroacetic acid solution*, *Filter paper* and *Standard test dilution*—Prepare as directed in the *Assay for protease activity* under *Pancreatin*.

Assay test dilution—Add an accurately weighed portion of the powder, prepared as directed in the *Assay for amylase activity*, equivalent to about 100 mg of pancreatin, to 100.0 mL of *Buffer solution*, and mix by shaking intermittently at room temperature for 25 minutes. Dilute quantitatively with *Buffer solution* to obtain a dilution that corresponds in activity to the *Standard test dilution*.

Procedure and Calculation of potency—Proceed as directed in the *Assay for protease activity* under *Pancreatin*.

Pancrelipase

» Pancrelipase is a substance containing enzymes, principally lipase, with amylase and protease, obtained from the pancreas of the hog, *Sus scrofa* Linné var. *domesticus* Gray (Fam. Suidae). It contains, in each mg, not less than 24 USP Units of lipase activity, not less than 100 USP Units of amylase activity, and not less than 100 USP Units of protease activity.

NOTE—One USP Unit of amylase activity is contained in the amount of pancrelipase that decomposes starch at an initial rate such that 0.16 μ Eq of glycosidic linkage is hydrolyzed per minute under the conditions of the *Assay for amylase activity*.

One USP Unit of lipase activity is contained in the amount of pancrelipase that liberates 1.0 μ Eq of acid per minute at pH 9.0 and 37° under the conditions of the *Assay for lipase activity*.

One USP Unit of protease activity is contained in the amount of pancrelipase that under the conditions of the *Assay for protease activity*, hydrolyzes casein at an initial rate such that there is liberated per minute an amount of peptides not precipitated by trichloroacetic acid that gives the same absorbance at 280 nm as 15 nmol of tyrosine.

Packaging and storage—Preserve in tight containers, preferably at a temperature not exceeding 25°.

Labeling—Label it to indicate lipase activity in USP Units.

USP Reference standards (11)—

USP Bile Salts RS

USP Pancreatin Amylase and Protease RS

USP Pancreatin Lipase RS

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

Loss on drying (731)—Dry it in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.

Fat—Place 2.0 g of Pancrelipase in a flask of about 50-mL capacity, add 20 mL of ether, insert the stopper, and set aside for 2 hours, mixing by rotating at frequent intervals. Decant the supernatant ether by means of a guiding rod into a plain filter about 7 cm in diameter, previously moistened with ether, and collect the filtrate in a tared beaker. Repeat the extraction with a 10-mL portion of ether, then with another 10-mL portion of ether, transfer the ether and the remainder of the Pancrelipase to the filter. Allow to drain, evaporate the ether spontaneously, and dry the residue at 105° for 2 hours: the residue of fat weighs not more than 100 mg (5.0%).

Assay for amylase activity (*Starch digestive power*)—

pH 6.8 Phosphate buffer—On the day of use, dissolve 13.6 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 14.2 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 51 mL of the monobasic potassium phosphate solution with 49 mL of the dibasic sodium phosphate solution. If necessary, adjust by the dropwise addition of the appropriate solution to a pH of 6.8.

Substrate solution—On the day of use, stir a portion of purified soluble starch equivalent to 2.0 g of dried substance

with 10 mL of water, and add this mixture to 160 mL of boiling water. Rinse the beaker with 10 mL of water, add it to the hot solution, and heat to boiling, with continuous mixing. Cool to room temperature, and add water to make 200 mL.

Standard preparation—Weigh accurately about 20 mg of USP Pancreatin Amylase and Protease RS into a suitable mortar. Add about 30 mL of pH 6.8 Phosphate buffer, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of pH 6.8 Phosphate buffer to a 50-mL volumetric flask, dilute with pH 6.8 phosphate buffer to volume, and mix. Calculate the activity, in USP Units of amylase activity per mL, of the resulting solution from the declared potency on the label of the Reference Standard.

Assay preparation—For Pancrelipase having about 4 times the amylase activity of the USP Pancreatin Amylase and Protease RS, weigh accurately about 10 mg of Pancrelipase into a suitable mortar. [NOTE—For Pancrelipase having a different amylase activity, weigh accurately the amount necessary to obtain an *Assay preparation* having amylase activity per mL corresponding approximately to that of the *Standard preparation*.] Add about 3 mL of pH 6.8 Phosphate buffer, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of pH 6.8 Phosphate buffer to a 100-mL volumetric flask, dilute with pH 6.8 Phosphate buffer to volume, and mix.

Procedure—Prepare four stoppered, 250-mL conical flasks, and mark them S, U, BS, and BU. Pipet into each flask 25 mL of *Substrate solution*, 10 mL of pH 6.8 Phosphate buffer, and 1 mL of sodium chloride solution (11.7 in 1000), insert the stoppers, and mix. Place the flasks in a water bath maintained at $25 \pm 0.1^\circ$, and allow them to equilibrate. To flasks BU and BS add 2 mL of 1 N hydrochloric acid, mix, and return the flasks to the water bath. To flasks U and BU add 1.0-mL portions of *Assay preparation*, and to flasks S and BS add 1.0 mL of *Standard preparation*. Mix each, and return the flasks to the water bath. After 10 minutes, accurately timed from the addition of the enzyme, add 2-mL portions of 1 N hydrochloric acid to flasks S and U, and mix. To each flask, with continuous stirring, add 10.0 mL of 0.1 N iodine VS, and immediately add 45 mL of 0.1 N sodium hydroxide. Place the flasks in the dark at a temperature between 15° and 25° for 15 minutes. To each flask add 4 mL of 2 N sulfuric acid, and titrate with 0.1 N sodium thiosulfate VS to the disappearance of the blue color. Calculate the amylase activity, in USP Units per mg, of the Pancrelipase taken by the formula:

$$100(C_S / W_U)(V_{BU} - V_U) / (V_{BS} - V_S)$$

in which C_S is the amylase activity of the *Standard preparation*, in USP Units per mL, W_U is the amount, in mg, of Pancrelipase taken, and V_U , V_S , V_{BU} , and V_{BS} are the volumes, in mL, of 0.1 N sodium thiosulfate consumed in the titration of the solutions in flasks U, S, BU, and BS, respectively.

Assay for lipase activity (*Fat digestive power*)—

Acacia solution—Centrifuge a solution of acacia (1 in 10) until clear. Use only the clear solution.

Olive oil substrate—Combine 165 mL of *Acacia solution*, 20 mL of olive oil, and 15 g of crushed ice in the cup of an electric blender. Cool the mixture in an ice bath to 5°, and homogenize at high speed for 15 minutes, intermittently cooling in an ice bath to prevent the temperature from exceeding 30°.

Test for suitability of mixing as follows. Place a drop of the homogenate on a microscope slide, and gently press a cover slide in place to spread the liquid. Examine the entire field under high power (43× objective lens and 5× ocular), using an eyepiece equipped with a calibrated micrometer. The substrate is satisfactory if 90% of the particles do not exceed 2 μ m in diameter and none exceeds 10 μ m in diameter.

Buffer solution—Dissolve 60 mg of tris(hydroxymethyl)aminomethane and 234 mg of sodium chloride in water to make 100 mL.

Bile salts solution—Prepare a solution to contain 80.0 mg of USP Bile Salts RS in each mL.

Standard test dilution—Suspend about 200 mg of USP Pancreatin Lipase RS, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 minutes, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based upon the declared potency on the label of the Reference Standard. Maintain the suspension at 4°, and mix before using. For each determination withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Assay test dilution—Suspend about 200 mg of Pancrelipase, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 minutes, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based upon the estimated potency of the test material. Maintain the suspension at 4°, and mix before using. For each determination withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Procedure—Mix 10.0 mL of *Olive oil substrate*, 8.0 mL of *Buffer solution*, 2.0 mL of *Bile salts solution*, and 9.0 mL of water in a jacketed glass vessel of about 50-mL capacity, the outer chamber of which is connected to a thermostatically controlled water bath. Cover the mixture, and stir continuously with a mechanical stirring device. With the mixture maintained at a temperature of $37 \pm 0.1^\circ$, add 0.1 N sodium hydroxide VS, from a microburet inserted through an opening in the cover, to adjust the pH to 9.20 potentiometrically using a calomel-glass electrode system. Add 1.0 mL of *Assay test dilution*, and then continue adding the 0.1 N sodium hydroxide VS for 5 minutes to maintain the pH at 9.0. Determine the volume of 0.1 N sodium hydroxide VS added after each minute.

In the same manner titrate 1.0 mL of *Standard test dilution*.

Calculation of potency—Plot the volume of 0.1 N sodium hydroxide VS titrated against time. Using only the points which fall on the straight-line segment of the curve, calculate the mean acidity released per minute by the test specimen and the Standard. Taking into consideration the dilution factors, calculate the lipase activity, in USP Units, of the Pancrelipase taken by comparison to the activity of the Reference Standard, using the lipase activity stated on the label of USP Pancreatin Lipase RS.

Assay for protease activity (Casein digestive power)—

Casein substrate—Place 1.25 g of finely powdered casein in a 100-mL conical flask containing 5 mL of water, shake to form a suspension, add 10 mL of 0.1 N sodium hydroxide, shake for 1 minute, add 50 mL of water, and shake for about 1 hour to dissolve the casein. If necessary, adjust to a pH of about 8, using 1 N sodium hydroxide or 1 N hydrochloric acid. Transfer the solution quantitatively to a 100-mL volumetric flask, dilute with water to volume, and mix. Use this substrate on the day it is prepared.

Buffer solution—Dissolve 6.8 g of monobasic potassium phosphate and 1.8 g of sodium hydroxide in 950 mL of water in a 1000-mL volumetric flask, adjust to a pH of 7.5 ± 0.2 , using 0.2 N sodium hydroxide, dilute with water to volume, and mix. Store this solution in a refrigerator.

Trichloroacetic acid solution—Dissolve 50 g of trichloroacetic acid in 1000 mL of water. This solution may be stored at room temperature.

Filter paper—Determine the suitability of the filter paper by filtering a 5-mL portion of *Trichloroacetic acid solution* through the paper and measuring the absorbance of the filtrate at 280 nm, using an unfiltered portion of the same *Trichloroacetic acid solution* as the blank: the absorbance is

not more than 0.04. If the absorbance is more than 0.04, wash the filter paper repeatedly with *Trichloroacetic acid solution* until the absorbance of the filtrate, determined as above, is not more than 0.04.

Standard test dilution—Add about 100 mg of USP Pancreatin Amylase and Protease RS, accurately weighed, to 100.0 mL of *Buffer solution*, and mix by shaking intermittently at room temperature for about 25 minutes. Dilute quantitatively with *Buffer solution* to produce a concentration of about 2.5 USP Units of protease activity per mL, based upon the declared potency on the label of the Reference Standard.

Assay test dilution—Weigh accurately about 100 mg of Pancrelipase into a suitable mortar. Add about 3 mL of *Buffer solution*, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *Buffer solution* to a 100-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Dilute quantitatively with *Buffer solution* to obtain a dilution that corresponds in activity to the *Standard test dilution*.

Procedure—Label test tubes in duplicate S_1 , S_2 , and S_3 for the standard series, and U for the sample. Pipet into tubes S_1 2.0 mL, into S_2 and U 1.5 mL, and into S_3 1.0 mL of *Buffer solution*. Pipet into tubes S_1 1.0 mL, into S_2 1.5 mL, and into S_3 2.0 mL of the *Standard test dilution*. Pipet into tubes U 1.5 mL of the *Assay test dilution*. Pipet into one tube each of S_1 , S_2 , S_3 , and U 5.0 mL of *Trichloroacetic acid solution*, and mix. Designate these tubes as S_{1B} , S_{2B} , S_{3B} , and U_B , respectively. Prepare a blank by mixing 3 mL of *Buffer solution* and 5 mL of *Trichloroacetic acid solution* in a separate test tube marked B . Place all the tubes in a 40° water bath, insert a glass stirring rod into each tube, and allow for temperature equilibration. At zero time, add to each tube, at timed intervals, 2.0 mL of the *Casein substrate*, preheated to the bath temperature, and mix. Sixty minutes, accurately timed, after the addition of the *Casein substrate* stop the reaction in tubes S_1 , S_2 , S_3 , and U by adding 5.0 mL of *Trichloroacetic acid solution* at the corresponding time intervals, stir, and remove all the tubes from the bath. Allow to stand at room temperature for 10 minutes for complete protein precipitation, and filter. The filtrates must be free from haze. Determine the absorbances of the filtrates, in 1-cm cells, at 280 nm, with a suitable spectrophotometer, using the filtrate from the blank (tube B) to set the instrument.

Calculation of potency—Correct the absorbance values for the filtrates from tubes S_1 , S_2 , and S_3 by subtracting the absorbance values for the filtrates from tubes S_{1B} , S_{2B} , and S_{3B} , respectively, and plot the corrected absorbance values against the corresponding volumes of the *Standard test dilution* used. From the curve, using the corrected absorbance value ($U - U_B$) for the Pancrelipase taken, and taking into consideration the dilution factors, calculate the protease activity, in USP Units, of the Pancrelipase taken by comparison with that of the Standard, using the protease activity stated on the label of USP Pancreatin Amylase and Protease RS.

Pancrelipase Capsules

» Pancrelipase Capsules contain an amount of Pancrelipase equivalent to not less than 90.0 percent and not more than 150.0 percent of the labeled lipase activity expressed in USP Units, the labeled activity being not less than 8000 USP Units per Capsule. They contain, in each Capsule, the pancrelipase equivalent of not less than 30,000 USP Units of amylase activity, and not less than 30,000 USP Units of protease activity.

Packaging and storage—Preserve in tight containers, preferably with a desiccant, at a temperature not exceeding 25°.

Labeling—Label the Capsules to indicate lipase activity in USP Units.

USP Reference standards (11)—

USP Bile Salts RS

USP Pancreatin Amylase and Protease RS

USP Pancreatin Lipase RS

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—Capsules meet the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

Loss on drying (731)—Dry the contents of 10 Capsules in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.

Assay—Weigh the contents of not less than 10 Capsules, and determine the average weight per capsule. Mix the combined contents, and proceed as directed for *Assay for amylase activity*, *Assay for lipase activity*, and *Assay for protease activity* under *Pancrelipase*.

Pancrelipase Delayed-Release Capsules

» Pancrelipase Delayed-Release Capsules contain an amount of Pancrelipase equivalent to not less than 90.0 percent and not more than 165.0 percent of the labeled lipase. It contains not less than 90.0 percent of the labeled activities of amylase and protease expressed in the respective USP Units.

Packaging and storage—Preserve in tight containers at controlled room temperature.

Labeling—Label the Capsules to indicate lipase, amylase, and protease activities in USP Units. The label also indicates that the Capsule contents are enteric-coated.

USP Reference standards (11)—

USP Bile Salts RS

USP Pancreatin Amylase and Protease RS

USP Pancreatin Lipase RS

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—Capsules meet the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

Dissolution (711)—

PART 1—

Medium: simulated gastric fluid TS, without enzyme; 800 mL.

Apparatus 1: 100 rpm.

Time: 60 minutes.

PART 2—

pH 6.0 phosphate buffer—Dissolve 8 g of sodium chloride and 36.8 g of monobasic potassium phosphate in 4000 mL of water. Adjust with 2 N sodium hydroxide to a pH of 6.0 ± 0.1.

Medium: pH 6.0 phosphate buffer; 800 mL.

Apparatus 2: 100 rpm.

Time: 30 minutes.

Standard solution—Proceed as directed under *Standard test dilution* in the *Assay for lipase activity* under *Pancrelipase*, except to use the *Dissolution Medium* in place of cold water.

Test solution—Empty the contents of 10 Capsules, and transfer an accurately weighed portion of the contents, equivalent to the concentration of USP Units of lipase activity per mL in the *Standard solution* (between 8 and 16 Units per mL), to *Apparatus 1*.

Procedure—Proceed according to the conditions for *Part 1*. After 1 hour, remove the baskets, and allow the excess *Dissolution Medium* to drain. Transfer the contents of each basket to the dissolution vessels in *Part 2* with the aid of a few mL of *Dissolution Medium*. Proceed according to the conditions for *Part 2*. After 30 minutes, remove a 10-mL portion of the solution under test, transfer to a test tube, and cool to 4°. Proceed as directed in the *Assay for lipase activity* under *Pancrelipase*.

Tolerances—Not less than 75% (Q) of the labeled USP Units of lipase activity per Capsule is dissolved.

Loss on drying (731)—Dry the contents of 10 Capsules in vacuum at 60° for 4 hours: the test specimen loses not more than 5.0% of its weight.

Assay—Weigh the contents of not less than 10 Capsules, and determine the average weight per Capsule. Grind the contents, mix the combined contents, and proceed as directed in the *Assay for lipase activity*, the *Assay for amylase activity*, and the *Assay for protease activity* under *Pancrelipase*.

Pancrelipase Tablets

» Pancrelipase Tablets contain an amount of Pancrelipase equivalent to not less than 90.0 percent and not more than 150.0 percent of the labeled lipase activity expressed in USP Units, the labeled activity being not less than 8000 USP Units per Tablet. They contain, in each Tablet, the pancrelipase equivalent of not less than 30,000 USP Units of amylase activity, and not less than 30,000 USP Units of protease activity.

Packaging and storage—Preserve in tight containers, preferably with a desiccant, at a temperature not exceeding 25°.

Labeling—Label the Tablets to indicate the lipase activity in USP Units.

USP Reference standards (11)—

USP Bile Salts RS

USP Pancreatin Amylase and Protease RS

USP Pancreatin Lipase RS

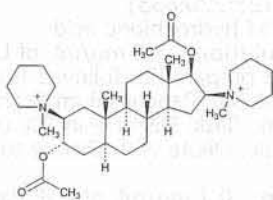
Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—Tablets meet the requirements of the test for absence of *Salmonella* species and *Escherichia coli*.

Disintegration (701): 75 minutes.

Loss on drying (731)—Dry about 5 g, accurately weighed, of finely ground Tablets in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.

Assay—Weigh and finely powder not less than 20 Tablets, avoiding the production of heat during the process, and proceed as directed for *Assay for amylase activity*, *Assay for lipase activity*, and *Assay for protease activity* under *Pancrelipase*.

Pancuronium Bromide



$C_{35}H_{60}Br_2N_2O_4$ 732.67

Piperidinium, 1,1'-[(2 β ,3 α ,5 α ,16 β ,17 β)-3,17-bis(acetyloxy)androstane-2,16-diyl]bis[1-methyl]-, dibromide; 1,1'-(3 α ,17 β -Dihydroxy-5 α -androstane-2 β ,16 β -ylene)bis[1-methylpiperidinium] dibromide diacetate; 2 β ,16 β -Dipiperidino-5 α -androstane-3 α ,17 β -diol diacetate dimethobromide [15500-66-0].

DEFINITION

Pancuronium Bromide contains NLT 98.0% and NMT 102.0% of pancuronium bromide ($C_{35}H_{60}Br_2N_2O_4$), calculated on the anhydrous basis.

IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- C. IDENTIFICATION TESTS—GENERAL, Bromide** (191): A solution (1 in 10) meets the requirements of the silver nitrate precipitate test for bromide.

ASSAY

PROCEDURE

Diluent: 0.0024 M hydrochloric acid

Mobile phase: Acetonitrile, methanol, and 0.024 M hydrochloric acid (125:200:675)

Standard stock solution: 1.0 mg/mL prepared as follows. Transfer the required quantity of USP Pancuronium Bromide RS to a suitable volumetric flask. Dissolve in 2% of the flask volume of acetonitrile, dilute with *Diluent* to volume, and sonicate for 3 min.

Standard solution: 0.1 mg/mL of USP Pancuronium Bromide RS in *Diluent*, from the *Standard stock solution*

Sample stock solution: 1.0 mg/mL prepared as follows. Transfer the required quantity of Pancuronium Bromide to a suitable volumetric flask. Dissolve in 2% of the flask volume of acetonitrile, dilute with *Diluent* to volume, and sonicate for 3 min.

Sample solution: 0.1 mg/mL of Pancuronium Bromide in *Diluent*, from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Conductivity with suppression

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Temperatures

Column: 35°

Detector: 40°

Suppressor: 4-mm cationic membrane suppressor or equivalent

Suppression solution: 0.15 M tetrabutylammonium hydroxide

Suppressor flow rate: 1 mL/min

Flow rate: 0.75 mL/min

Injection volume: 25 μ L

Run time: 2 times the retention time of pancuronium

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pancuronium bromide ($C_{35}H_{60}Br_2N_2O_4$) in the portion of Pancuronium Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Pancuronium Bromide RS in the *Standard solution* (mg/mL)

C_U = concentration of Pancuronium Bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

RESIDUE ON IGNITION (281): NMT 0.1%

ORGANIC IMPURITIES

Diluent, Mobile phase, Standard stock solution, and Chromatographic system: Proceed as directed in the *Assay*.

System suitability solution: 1 mg/mL of USP Pancuronium Bromide RS and 0.02 mg/mL each of USP Pancuronium Bromide Related Compound A RS, USP Pancuronium Bromide Related Compound B RS, USP Pancuronium Bromide Related Compound C RS, and USP Vecuronium Bromide RS, prepared as follows. Transfer the required amounts of the individual components to a suitable volumetric flask. Dissolve in 2% of the flask volume of acetonitrile, dilute with *Diluent* to volume, and sonicate for 3 min.

Standard solution: 0.01 mg/mL of USP Pancuronium Bromide RS in *Diluent*, from the *Standard stock solution*

Sample solution: 1.0 mg/mL prepared as follows.

Transfer the required quantity of Pancuronium Bromide to a suitable volumetric flask. Dissolve in 2% of the flask volume of acetonitrile, dilute with *Diluent* to volume, and sonicate for 3 min.

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between pancuronium related compound B and pancuronium related compound A, and NLT 1.5 between the pancuronium related compound C and vecuronium peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity, including any unspecified impurity, in the portion of Pancuronium Bromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of pancuronium from the *Standard solution*

- C_s = concentration of USP Pancuronium Bromide RS in the *Standard solution* (mg/mL)
 C_u = concentration of Pancuronium Bromide in the *Sample solution* (mg/mL)
 F = relative response factor (see Table 1)
 Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Pancuronium related compound B	0.73	1.0	0.1
Pancuronium related compound A	0.81	1.0	0.1
Vecuronium related compound F ^a	0.9	—	—
Pancuronium	1.0	—	—
Pancuronium related compound C	1.39	1.0	0.1
Vecuronium	1.53	0.48	1.0
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

^a Piperidinium, 1-[(2,3,5,16,17)-17-acetyloxy-3-hydroxy-2-(1-piperidinyl)androstane-16-yl]-1-methyl. This impurity is an acid degradation product of vecuronium bromide and not that of pancuronium bromide.

SPECIFIC TESTS

- OPTICAL ROTATION, Specific Rotation (781S)**
Sample solution: 30 mg/mL in water
Acceptance criteria: +39° to +43°
- WATER DETERMINATION, Method I (921):** NMT 8.0%

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and moisture.
- USP REFERENCE STANDARDS (11)**
 USP Pancuronium Bromide RS
 USP Pancuronium Bromide Related Compound A RS
 1,1'-(3 α ,17 β -Dihydroxy-5 α -androstane-2 β ,16 β -ylene) bis(1-methylpiperidinium) dibromide.
 $C_{31}H_{56}Br_2N_2O_2$ 648.60
 USP Pancuronium Bromide Related Compound B RS
 1,1'-(17 β -Acetoxy-3 α -hydroxy-5 α -androstane-2 β ,16 β -ylene) bis(1-methylpiperidinium) dibromide.
 $C_{33}H_{58}Br_2N_2O_3$ 690.63
 USP Pancuronium Bromide Related Compound C RS
 1,1'-(3 α -Acetoxy-17 β -hydroxy-5 α -androstane-2 β ,16 β -ylene) bis(1-methylpiperidinium) dibromide.
 $C_{33}H_{58}Br_2N_2O_3$ 690.63
 USP Vecuronium Bromide RS

Pancuronium Bromide Injection

DEFINITION

Pancuronium Bromide Injection is a sterile solution containing NLT 92.0% and NMT 105.0% of the labeled amount of pancuronium bromide ($C_{35}H_{60}Br_2N_2O_4$) in Water for Injection. It contains a suitable tonicity-adjusting agent.

IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Mobile phase: Acetonitrile, methanol, and 0.024 M hydrochloric acid (125:220:655)

Diluent: 0.0024 M hydrochloric acid

Standard stock solution: 1.0 mg/mL of USP Pancuronium Bromide RS prepared as follows. Transfer the required quantity of USP Pancuronium Bromide RS to a suitable volumetric flask. Dissolve in 2% of the flask volume of acetonitrile. Dilute with *Diluent* to volume. Sonicate for 3 min.

Standard solution: 0.1 mg/mL of USP Pancuronium Bromide RS from the *Standard stock solution* and *Diluent*

Sample solution: Nominally 0.1 mg/mL of pancuronium bromide from a suitable volume of Injection in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Conductivity with suppression

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Temperatures

Detector: 40°

Column: 35°

Suppressor: 4-mm cationic membrane suppressor or equivalent

Suppression solution: 0.15 M tetrabutylammonium hydroxide

Suppressor flow rate: 1 mL/min

Flow rate: 0.75 mL/min

Injection volume: 25 μ L

Run time: 2 times the retention time of pancuronium

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pancuronium bromide ($C_{35}H_{60}Br_2N_2O_4$) in the portion of Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Pancuronium Bromide RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of pancuronium bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 92.0%–105.0%

IMPURITIES

ORGANIC IMPURITIES

Diluent, Standard stock solution, and Chromatographic system: Proceed as directed in the Assay.

Mobile phase: Acetonitrile, methanol, and 0.024 M hydrochloric acid (125:180:695)

System suitability solution: 1 mg/mL of USP Pancuronium Bromide RS and 0.02 mg/mL each of USP Pancuronium Bromide Related Compound A RS, USP Pancuronium Bromide Related Compound B RS, USP Pancuronium Bromide Related Compound C RS, and USP Vecuronium Bromide RS prepared as follows. Transfer the required amounts of the individual components to a suitable volumetric flask. Dissolve in 2% of the flask volume of acetonitrile, and dilute with *Diluent* to volume. Sonicate for 3 min.

Standard solution: 0.02 mg/mL of USP Pancuronium Bromide RS from the *Standard stock solution* and *Diluent*

Sample solution: Nominally 1.0 mg/mL of pancuronium bromide from a suitable volume of Injection in *Diluent*

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between pancuronium related compound B and pancuronium related compound A; NLT 1.5 between pancuronium related compound C and vecuronium peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity as well as any unspecified impurity in the portion of Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of each impurity from the *Sample solution*

r_s = peak response of the pancuronium peak from the *Standard solution*

C_s = concentration of USP Pancuronium Bromide RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of pancuronium bromide in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pancuronium related compound B	0.70	3.0
Pancuronium related compound A	0.78	0.4
Vecuronium related compound F ^a	0.90	—
Pancuronium	1.0	—
Pancuronium related compound C	1.47	2.0
Vecuronium ^b	1.69	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	5.0

^a Piperidinium, 1-[(2,3,5,16,17)-17-acetyloxy-3-hydroxy-2-(1-piperidinyl)androstano-16-yl]-1-methyl. This impurity is the acid degradation product of vecuronium bromide and may be present only in the *System suitability solution*.

^b This process impurity is included for peak identification purposes only and is controlled in the drug substance. This is not included in the total impurities.

SPECIFIC TESTS

- **PH (791):** 3.8–4.2
- **PARTICULATE MATTER IN INJECTIONS (788):** It meets the requirements for small-volume injections.
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 50 USP Endotoxin Units/mg of pancuronium bromide.
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS (1):** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, single-dose or multiple-dose containers for injections, as described in *Packaging and Storage Requirements (659)*, *Injection Packaging*, preferably of Type 1 glass. Store in a refrigerator between 2° and 8°, protected from light.

- **USP REFERENCE STANDARDS (11)**

USP Pancuronium Bromide RS

USP Pancuronium Bromide Related Compound A RS
1,1'-(3 α ,17 β -Dihydroxy-5 α -androstano-2 β ,16 β -ylene) bis(1-methylpiperidinium) dibromide.

C₃₁H₅₆Br₂N₂O₂ 648.60

USP Pancuronium Bromide Related Compound B RS
1,1'-(17 β -Acetoxy-3 α -hydroxy-5 α -androstano-2 β ,16 β -ylene) bis(1-methylpiperidinium) dibromide.

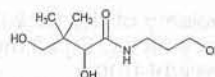
C₃₃H₅₈Br₂N₂O₃ 690.63

USP Pancuronium Bromide Related Compound C RS
1,1'-(3 α -Acetoxy-17 β -hydroxy-5 α -androstano-2 β ,16 β -ylene) bis(1-methylpiperidinium) dibromide.

C₃₃H₅₈Br₂N₂O₃ 690.63

USP Vecuronium Bromide RS

Panthenol



C₉H₁₉NO₄

205.25

Butanamide, 2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethyl-, (±)-;

(±)-2,4-Dihydroxy-N-(3-hydroxypropyl)-3,3-dimethylbutanamide;

(±)-Pantothenyl alcohol [16485-10-2].

DEFINITION

Panthenol is a racemic mixture of the dextrorotatory and levorotatory isomers of panthenol. It contains NLT 99.0% and NMT 102.0% of racemic panthenol (C₉H₁₉NO₄), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**

- **B.**

Sample solution: 100 mg/mL of Panthenol

Analysis: To 1 mL of the *Sample solution* add 5 mL of 1 N sodium hydroxide and 1 drop of cupric sulfate TS, and shake vigorously.

Acceptance criteria: A deep blue color develops.

- **C.**

Sample solution: 10 mg/mL of Panthenol

Analysis: To 1 mL of the *Sample solution* add 1 mL of 1 N hydrochloric acid, and heat on a steam bath for 30 min. Cool, add 100 mg of hydroxylamine hydrochloride, and add 5 mL of 1 N sodium hydroxide. Allow to stand for 5 min, then adjust with 1 N hydrochloric acid to a pH of 2.5–3.0, and add 1 drop of ferric chloride TS.

Acceptance criteria: A purplish red color develops.

ASSAY

- **PROCEDURE**

0.1 M potassium biphthalate: Transfer 20.42 g of potassium biphthalate into a 1000-mL volumetric flask, and add sufficient glacial acetic acid to dissolve. If necessary, warm the mixture on a steam bath to achieve complete solution, observing precautions against absorption of moisture. Cool to room temperature, and dilute with glacial acetic acid to volume.

Sample: 400 mg of Panthenol

Blank: Proceed as directed in the *Analysis*, omitting the *Sample*.

Titrimetric system

(See *Titrimetry (541)*.)

Mode: Residual titration
Titrant: 0.1 N perchloric acid VS
Back-titrant: 0.1 M potassium biphthalate
Endpoint detection: Visual

Analysis: Transfer the *Sample* to a 300-mL flask fitted to a reflux condenser by means of a standard-taper glass joint, add 50.0 mL of *Titrant*, and reflux for 5 h. Cool, observing precautions to prevent atmospheric moisture from entering the condenser, and rinse the condenser with glacial acetic acid, collecting the rinsings in the flask. To the flask add 5 drops of crystal violet TS, and titrate with the *Back-titrant* to a blue-green endpoint. Perform the *Blank* determination.

Calculate the percentage of panthenol ($C_5H_{19}NO_4$) in the *Sample* taken:

$$\text{Result} = \{[(V_B - V_S) \times M \times F] / W\} \times 100$$

V_B = *Back-titrant* volume consumed by the *Blank* (mL)

V_S = *Back-titrant* volume consumed by the *Sample* (mL)

M = actual molarity of the *Back-titrant* (mM/mL)

F = equivalency factor, 205.3 mg/M

W = *Sample* weight (mg)

Acceptance criteria: 99.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **LIMIT OF AMINOPROPANOL**

Sample: 10 g of Panthenol

Blank: 25 mL of water

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.01 N sulfuric acid VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 25 mL of water, and add bromothymol blue TS. Titrate with the *Titrant* to a yellow endpoint. Perform the *Blank* determination.

Calculate the percentage of aminopropanol in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F] / W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 75.11 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: NMT 0.10%

SPECIFIC TESTS

• **MELTING RANGE, Class I** (741): 64.5°–68.5°

• **OPTICAL ROTATION, Specific Rotation** (781S)

Sample solution: 50 mg/mL in water

Acceptance criteria: –0.05° to +0.05°

• **LOSS ON DRYING** (731): Dry a sample in a vacuum over phosphorus pentoxide at 56° for 4 h; it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Racemic Panthenol RS

follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).¹

Pantoprazole Sodium	200 mg
Sodium Bicarbonate 8.4% Injection, a sufficient quantity to make	100 mL

Calculate the required quantity of each ingredient for the total amount to be prepared. Remove the trademark imprint from the tablets by gently rubbing on a paper towel that has been dampened with Alcohol, USP. Allow the tablets to air-dry for a few min. Triturate the tablets to a coarse powder by using a mortar and pestle, and transfer to a calibrated bottle. Add 50 mL of *Sodium Bicarbonate 8.4% Injection* and agitate until the coating is dissolved. Add sufficient *Sodium Bicarbonate 8.4% Injection* to bring the final volume to 100 mL and mix well until the powder is uniformly suspended.

[NOTE—If the imprint is not properly removed, the pharmaceutical elegance of the final product will be compromised by the presence of flecks of dark material.]

ASSAY

PROCEDURE

Mobile phase: Acetonitrile and 50 mM dibasic potassium phosphate (2:3). Adjust with phosphoric acid to a pH of 7.0. Make adjustments if necessary.

Standard stock solution: 1.0 mg/mL of USP Pantoprazole Sodium RS in water

Standard solution: Transfer 1.5 mL of *Standard stock solution* to a 100-mL volumetric flask, and dilute with water to volume in order to obtain a solution containing about 15 µg/mL of pantoprazole sodium. Pass through a suitable filter of 0.22-µm pore size.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Accurately pipet 3.75 mL of Oral Suspension to a 50-mL volumetric flask. Add 25 mL of water to the flask, and place on an orbital shaker for 20 min. Dilute with water to volume. Take a 5-mL portion of the diluted sample and further dilute with water to 50 mL to obtain a solution with a nominal concentration of 15 µg/mL of pantoprazole sodium. Pass through a suitable filter of 0.22-µm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.6 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for the pantoprazole peak is about 2.6 min.]

Suitability requirements

Relative standard deviation: NMT 1.0% for the replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of $C_{16}H_{14}F_2N_3NaO_4S \cdot 1.5 H_2O$ in the volume of Oral Suspension taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of pantoprazole sodium in the *Standard solution* (µg/mL)

C_U = nominal concentration of pantoprazole sodium in the *Sample solution* (µg/mL)

¹ This formula is frequently used for home health patients with feeding tubes who have been discharged from hospitals. The goal of the drug is to neutralize the acidity of the stomach.

Pantoprazole Oral Suspension

DEFINITION

Pantoprazole Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled content of pantoprazole sodium. Prepare Pantoprazole Oral Suspension, 2 mg/mL, as

Acceptance criteria: 90.0%–110.0%

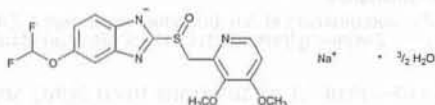
SPECIFIC TESTS

- **pH (791):** Between 7.9 and 8.3

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled cold temperature.
- **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 14 days after the date on which it was compounded when stored at controlled cold temperature
- **USP REFERENCE STANDARDS (11)**
USP Pantoprazole Sodium RS

Pantoprazole Sodium



$C_{16}H_{14}F_2N_3NaO_4S \cdot 1.5H_2O$ 432.37
1*H*-Benzimidazole, 5-(difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridyl)methyl]sulfonyl-, sodium salt, hydrate (2:3).
5-(Difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridyl)methyl]sulfonyl]benzimidazole, sodium salt, sesquihydrate [164579-32-2].

» Pantoprazole Sodium contains not less than 98.0 percent and not more than 102.0 percent of $C_{16}H_{14}F_2N_3NaO_4S$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed, light-resistant containers. Store at room temperature.

Labeling—If a test for *Related compounds* other than *Test 1* is used, then the labeling states the test with which the article complies.

USP Reference standards (11)—

USP Pantoprazole Sodium RS

USP Pantoprazole Related Compound A RS

5-(Difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridinyl)methyl]sulfonyl]-1*H*-benzimidazole.

$C_{16}H_{14}F_2N_3O_5S$ 399.37

USP Pantoprazole Related Compound B RS

5-(Difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridinyl)methyl]thio]-1*H*-benzimidazole.

$C_{16}H_{14}F_2N_3O_3S$ 367.37

USP Pantoprazole Related Compound C RS

5-(Difluoromethoxy)-1*H*-benzimidazole-2-thiol.

$C_8H_6F_2N_2OS$ 216.21

USP Pantoprazole Related Compound D and F Mixture RS

A mixture of 5-(difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfonyl]-1-methyl-1*H*-benzimidazole and 6-(difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfonyl]-1-methyl-1*H*-benzimidazole.

$C_{17}H_{17}F_2N_3O_4S$ 397.40

USP Pantoprazole Related Compound E RS

A mixture of the stereoisomers of 6,6'-bis(difluoromethoxy)-2,2'-bis[(3,4-dimethoxypyridin-2-yl)methyl]sulfonyl]-1*H*,1'-*H*-5,5'-bibenzimidazolyl.

$C_{32}H_{28}F_4N_6O_8S_2$ 764.74

Identification—

A: *Infrared Absorption* (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the

chromatogram of the *Standard preparation*, as obtained in the *Assay*.

C: It meets the requirements of the pyroantimonate precipitate test for *Sodium* (191).

Water Determination, Method I (921): between 5.0% and 8.0%.

Delete the following:

• **Heavy metals, Method II (231):** not more than 0.002%.

• (Official 1-Jan-2018)

Related compounds—[NOTE—On the basis of the synthetic route, perform either *Test 1* or *Test 2*. *Test 2* is recommended when impurities C, D, E, and F are potential related compounds.]

TEST 1—[NOTE—Protect all solutions from light, and use amber autosampler vials and low-actinic glassware.]

Diluent, Mobile phase, System suitability preparation, and Chromatographic system—Proceed as directed in the *Assay*.

Standard solution—Transfer about 20 mg of USP Pantoprazole Sodium RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 to 10 mL of a mixture of acetonitrile and water (1:1), and dilute with *Diluent* to volume. Further dilute with *Diluent* quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.0004 mg per mL.

Test solution—Transfer about 20 mg of Pantoprazole Sodium, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 to 10 mL of a mixture of acetonitrile and water (1:1), dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—Prepare as directed in the *Assay*. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*. Identify the components on the basis of their relative retention times (*Table 1*): the resolution, *R*, between the pantoprazole related compound A and pantoprazole peaks is not less than 10.0.

Table 1

Name	Relative Retention Time	Limit (%)
Pantoprazole related compound A ¹	0.52	0.20
Pantoprazole sodium	1.0	N/A
Pantoprazole related compound B ²	1.7	0.15
Any other individual impurity	—	0.10
Total impurities	—	0.5

¹ 5-(Difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridyl)methyl]sulfonyl]-1*H*-benzimidazole.

² 5-(Difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridyl)methyl]thio]-1*H*-benzimidazole.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Pantoprazole Sodium taken by the formula:

$$100(C_s / C_r)(r_i / r_s)$$

in which C_s and C_r are the concentrations, in mg per mL, of pantoprazole sodium in the *Standard solution* and the *Test solution*, respectively; r_i is the peak response of each impurity obtained from the *Test solution*; and r_s is the pantoprazole peak response obtained from the *Standard solution*. The reporting level for impurities is 0.05%.

TEST 2—

Diluent—Prepare a mixture of acetonitrile and 0.001 N sodium hydroxide solution (50:50).

Standard solution—Dissolve an accurately weighed quantity of USP Pantoprazole Sodium RS in *Diluent*, and dilute quantitatively to obtain a solution having a known concentration of about 0.03 mg per mL.

Test solution—Prepare a solution of Pantoprazole Sodium in *Diluent* having a known concentration of about 0.46 mg per mL.

System suitability solution—Dissolve suitable amounts of USP Pantoprazole Sodium RS, USP Pantoprazole Related Compound A RS, USP Pantoprazole Related Compound B RS, USP Pantoprazole Related Compound C RS, USP Pantoprazole Related Compound D and F Mixture RS, and USP Pantoprazole Related Compound E RS in *Diluent* to obtain a solution containing about 0.46 mg of pantoprazole sodium per mL and about 1.3 µg each of related compounds A, B, C, and E per mL, and about 1.3 µg of the D and F mixture per mL.

Solution A—Prepare a solution of dibasic potassium phosphate (1.74 g/L) adjusted with a solution of phosphoric acid (330 g/L) to a pH of 7.00 ± 0.05.

Solution B—Use acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed below for *Chromatographic system*. Make adjustments as necessary (see *System Suitability* under *Chromatography* (621)).

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with either a programmable variable wavelength detector or two separate detectors capable of monitoring at 290 nm and at 305 nm, and a 4-mm × 12.5-cm column that contains 5-µm packing L1. The column temperature is maintained at 40°. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–40	80→20	20→80	linear gradient
40–45	20→80	80→20	linear gradient
45–55	80	20	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses at 290 nm as directed for *Procedure*. Identify the components based on relative retention times (*Table 2*): the resolution, *R*, between pantoprazole related compound E and pantoprazole related compounds D and F is not less than 1.5. Chromatograph the *Standard solution* at 290 nm, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms at 290 nm and 305 nm, and measure the responses for the major peaks.

[NOTE—Pantoprazole related compound C is monitored using a wavelength of 305 nm, and all other compounds are monitored at 290 nm.] Calculate the percentage of each impurity in the portion of Pantoprazole Sodium taken by the formula:

$$100 (1 / F)(C_s / C_t)(r_i / r_s)$$

in which *C_s* is the concentration, in mg per mL, of pantoprazole sodium in the *Standard solution*; *C_t* is the concentration, in mg per mL of Pantoprazole Sodium in the *Test solution*; *F* is the response factor of an individual pantoprazole related compound relative to the response of pantoprazole sodium (*Table 2*); *r_i* is the peak response of each impurity obtained from the *Test solution*; and *r_s* is the pantoprazole peak response obtained from the *Standard solution*. The reporting level for impurities is 0.05%.

Table 2

Impurity Name	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Related compound A	0.9	1.0	0.20
Related compound B	1.5	1.0	0.15
Related compound C ¹	0.6	3.3	0.10 ²
Related compounds D ³ and F ⁵	1.2	1.0	0.20 ⁴
Related compound E ⁶	1.3	1.0	0.10
Any other individual impurity	—	—	0.10
Total impurities	—	—	0.5

¹ 5-(Difluoromethoxy)-1*H*-benzimidazole-2-thiol.

² At 305 nm.

³ 5-(Difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfanyl]-1-methyl-1*H*-benzimidazole.

⁴ Impurities D and F are not fully resolved and should be integrated together.

⁵ 6-(Difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfanyl]-1-methyl-1*H*-benzimidazole.

⁶ Mixture of the stereoisomers of 6,6'-bis(difluoromethoxy)-2,2'-bis[[[3,4-dimethoxypyridin-2-yl)methyl]sulfanyl]-1*H*,1'*H*-5,5'-bibenzimidazolyl.

Assay—[NOTE—Protect all solutions from light, and use amber autosampler vials and low-actinic glassware.]

Ammonium phosphate buffer—Dissolve 1.32 g of dibasic ammonium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 7.5.

Acetonitrile-methanol mixture—Prepare a mixture of acetonitrile and methanol (7:3).

Solution A—Use a filtered and degassed mixture of *Ammonium phosphate buffer* and *Acetonitrile-methanol mixture* (85:15).

Solution B—Use *Acetonitrile-methanol mixture*.

Diluent—Transfer 25 mL of ammonium hydroxide to a suitable container, and dilute with water to 500 mL.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability preparation—Dissolve suitable amounts of USP Pantoprazole Sodium RS, USP Pantoprazole Related Compound A RS, and USP Pantoprazole Related Compound B RS in a mixture of acetonitrile and water (1:1) to obtain a solution having about 0.5 mg of each component per mL. Transfer 1 mL of this solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Standard preparation—Transfer about 20 mg of USP Pantoprazole Sodium RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 to 10 mL of a mixture of acetonitrile and water (1:1), and dilute with *Diluent* to volume. Further dilute with *Diluent* quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.06 mg per mL.

Assay preparation—Transfer about 20 mg of Pantoprazole Sodium, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 to 10 mL of a mixture of acetonitrile and water (1:1), and dilute with *Diluent* to volume. Further dilute with *Diluent* quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.06 mg per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 285-nm detector and 3.9-mm × 15-cm column that contains 4-µm packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at 30°, and the autosampler temperature is maintained at 4°. The chromatograph is programmed as follows:

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–10	86	14	isocratic
10–35	86→42	14→58	linear gradient
35–36	42→86	58→14	linear gradient
36–46	86	14	re-equilibration

Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*. Identify the components based on their relative retention times (Table 1): the resolution, R , between the pantoprazole related compound A and pantoprazole peaks is not less than 10.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{16}H_{14}F_2N_3NaO_4S$ in the portion of Pantoprazole Sodium taken by the formula:

$$100(C_s / C_u)(r_u / r_s)$$

in which C_s and C_u are the concentrations, in mg per mL, of pantoprazole sodium in the *Standard preparation* and the *Assay preparation*, respectively; and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pantoprazole Sodium Delayed-Release Tablets

DEFINITION

Pantoprazole Sodium Delayed-Release Tablets contain an amount of Pantoprazole Sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of pantoprazole ($C_{16}H_{14}F_2N_3O_4S$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Solution A: Dissolve 3.85 g of ammonium acetate and 1.1 g of tetrabutylammonium hydrogen sulfate in 1 L of water, and adjust with ammonium hydroxide solution diluted 1:1 with water to a pH of 7.9.

Diluent: Mixture of acetonitrile and 0.02 N sodium hydroxide (1:1)

Mobile phase: Prepare a mixture of acetonitrile and *Solution A* (35:65).

Standard solution: Transfer a weighed quantity of USP Pantoprazole Sodium RS to a suitable volumetric flask, add 0.02 N sodium hydroxide to about 60% of the final volume, sonicate for 5 min to dissolve, add about 2% of acetonitrile, and dilute with 0.02 N sodium hydroxide to volume to obtain a solution having a known concentration of about 0.2 mg/mL of pantoprazole sodium.

System suitability solution: Prepare a solution in 0.02 N sodium hydroxide, using sonication if necessary, containing about 0.2 mg/mL of pantoprazole sodium and about 0.0004 mg/mL each of USP Pantoprazole Related Compound A RS and USP Pantoprazole Related Compound B RS.

Sample solution: Transfer 5 Tablets into a suitable volumetric flask. [NOTE—Use 50- or 100-mL volumetric flasks for Tablets containing 20 or 40 mg of pantoprazole per Tablet, respectively.] Add *Diluent* to about 60% of the final volume, shake mechanically for about 60 min, and dilute with *Diluent* to volume. Pass through a suitable filter, and dilute the filtrate with 0.02 N sodium hydroxide to obtain a solution having a known concentration of about 0.2 mg/mL of pantoprazole, based on the label claim.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection size: 20 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 3 between pantoprazole and pantoprazole related compound A, *System suitability solution*

Tailing factor: NMT 2.0, *System suitability solution*

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{16}H_{14}F_2N_3O_4S$ in the portion of Tablets taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times (M_{r1} / M_{r2}) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Pantoprazole Sodium RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of pantoprazole in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of pantoprazole, 383.37

M_{r2} = molecular weight of pantoprazole sodium, 405.35

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Test 1: Proceed as directed for *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*, *Method B*, *Procedure*.

Acid stage

Acid stage medium: 0.1 N hydrochloric acid; 1000 mL

Apparatus 2: 75 rpm

Time: 120 min

Determine the amount of pantoprazole dissolved in the *Acid stage* using the following procedure.

Sample solution: After 120 min, withdraw an aliquot, pass through a suitable filter of 0.45- μ m pore size, and immediately dilute a portion of the filtrate by a factor of 2 with 0.5 N sodium hydroxide. Transfer the Tablets to the vessels containing the *Buffer stage medium*.

Diluent: Prepare a mixture of pH 6.8 phosphate buffer and 0.5 N sodium hydroxide (1:1).

Mobile phase: Acetonitrile, triethylamine, and water (40:1:60). Adjust with phosphoric acid to a pH of 7.0 \pm 0.05.

Standard stock solution: Transfer about 20 mg of USP Pantoprazole Sodium RS to a 50-mL volumetric flask. Add about 30 mL of 0.02 N sodium hydroxide, and sonicate until dissolved. Add 2 mL of acetonitrile, and dilute with 0.02 N sodium hydroxide to volume.

Standard solution: Transfer 1.0 mL of the *Standard stock solution* to a 20-mL volumetric flask, and dilute with *Diluent* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 4.6-mm × 7.5-cm; 3-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection size: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount of pantoprazole released, as a percentage, in the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (100/L)$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of pantoprazole sodium in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of pantoprazole, 383.37

M_{r2} = molecular weight of pantoprazole sodium, 405.35

V = volume of *Medium*, 1000 mL

L = Tablet label claim (mg)

Tolerances: NMT 10% of the labeled amount of pantoprazole is dissolved.

Buffer stage

Buffer stage medium: pH 6.8 phosphate buffer; 1000 mL

Apparatus 2: 75 rpm

Time: 30 min

Analysis: After 30 min, withdraw an aliquot, pass through a suitable filter of 0.45-μm pore size, and immediately dilute a portion of the filtrate by a factor of 2 with 0.5 N sodium hydroxide. Determine the amount of pantoprazole dissolved in the *Buffer stage* using the same procedure as for the *Acid stage*.

Tolerances: NLT 75% (Q) of the labeled amount of pantoprazole is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*. Proceed as directed for *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*, *Method B*, *Procedure*.

Acid stage

Acid stage medium: 0.1 N hydrochloric acid; 1000 mL

Apparatus 2: 100 rpm

Time: 2 h

Standard stock solution: Transfer a quantity of USP Pantoprazole Sodium RS to a suitable volumetric flask. Dissolve first in 0.1 N sodium hydroxide, using 10% of the final volume, then dilute with pH 6.8 phosphate buffer to volume, to obtain a solution having a known concentration of about 0.46 mg of pantoprazole sodium per mL. Mix well until a clear solution is obtained. Calculate the concentration in mg of pantoprazole per mL, the molecular weights of pantoprazole and pantoprazole sodium being 383.37 and 405.35, respectively.

Acid stage standard solution: Dilute an appropriate volume of the *Standard stock solution* to 1 L with *Acid stage medium* in such a way as to obtain a final concentration of about 10% of the Tablet label claim per L.

Sample solution: Pass a portion of the solution under test through a suitable filter of 10-μm pore size.

Analysis: Determine the amount of pantoprazole dissolved by using UV absorption at the wavelength of about 305 nm on portions of the *Sample solution* in comparison to the *Acid stage working standard solution* using a 4-cm path length cell and *Acid stage medium* as blank. Drain the *Acid stage medium* from each vessel and replace with *Buffer stage medium*. Calculate the amount of pantoprazole dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (100/L)$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of pantoprazole in the *Acid stage standard solution* (mg/mL)

V = volume of *Medium*, 1000 mL

L = Tablet label claim of pantoprazole (mg)

Tolerances: NMT 10% of the labeled amount of pantoprazole is dissolved.

Buffer stage

Buffer stage medium: pH 6.8 phosphate buffer; 1000 mL

Apparatus 2: 100 rpm

Time: 45 min

Buffer stage standard solution: Dilute an appropriate volume of the *Standard stock solution* as described under *Acid stage* to 250 mL with *Buffer stage medium* in such a way as to obtain a final concentration of about 100% of the Tablet label claim per L.

Sample solution: Pass a portion of the solution under test through a suitable filter of 10-μm pore size.

Analysis: Determine the amount of pantoprazole dissolved by using UV absorption at the wavelength of maximum absorbance at about 288 nm on portions of the *Sample solution* in comparison to *Buffer stage standard solution* using a 0.5-cm path length cell and *Buffer stage medium* as blank.

Calculate the amount of pantoprazole dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (100/L)$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Buffer stage standard solution*

C_S = concentration of pantoprazole in the *Buffer stage standard solution* (mg/mL)

V = volume of the *Buffer stage medium*, 1000 mL

L = Tablet label claim (mg)

Tolerances: NLT 75% (Q) of the labeled amount of pantoprazole is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*. Proceed as directed for *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*, *Method B*, *Procedure*.

Acid stage

Acid stage medium: 0.1 N hydrochloric acid; 1000 mL

Apparatus 2: 100 rpm

Time: 2 h

Dilute ammonia solution: Transfer 40 mL of strong ammonia solution to a 100-mL volumetric flask, and dilute with water to volume.

Buffer solution: Transfer 1.5 g of ammonium acetate to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Adjust the pH with *Dilute ammonia solution* to 7.0 ± 0.1.

Mobile phase: Methanol and *Buffer solution* (2:3)

Standard solution: 0.4 mg/mL. Transfer a quantity of USP Pantoprazole Sodium RS to a suitable volumetric flask, add 10% of the final volume of methanol, sonicate, and dilute with *Mobile phase* to volume.

Sample solution: After 2 h in the *Acid stage medium*, decant the medium from the vessel, remove the Tablet from the vessel, and dry it with tissue paper.

Transfer the Tablet to a suitable volumetric flask, add 20% of the final volume of methanol, and sonicate for about 20 min. Dilute with *Mobile phase* to volume to obtain a final concentration of about 0.4 mg/mL of pantoprazole. Mix well, centrifuge, and use the supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Temperature

Column: Ambient

Autosampler: 4°

Flow rate: 1.5 mL/min

Injection size: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 7500 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount of pantoprazole released, as a percentage, in the *Acid stage*:

$$\text{Result} = A - [(r_U/r_S) \times C_S \times D_U \times (M_{r1}/M_{r2}) \times (100/L)]$$

A = percentage of pantoprazole as determined in the *Assay*

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of pantoprazole sodium in the *Standard solution* (mg/mL)

D_U = dilution factor of the *Sample solution*

M_{r1} = molecular weight of pantoprazole, 383.37

M_{r2} = molecular weight of pantoprazole sodium, 405.35

L = Tablet-label claim (mg)

Tolerances: NMT 10% of the labeled amount of pantoprazole is dissolved.

Buffer stage

Buffer stage medium: pH 6.8 phosphate buffer; 1000 mL

Apparatus 2: 100 rpm

Time: 45 min

Standard solution: Further dilute an appropriate volume of the *Standard solution* prepared in the *Acid stage* with *Buffer stage medium* to obtain a solution having a known concentration of about 0.04 mg/mL.

Sample solution: Transfer a separate Tablet to the vessel containing *Acid stage medium*, and proceed as directed for the *Acid stage*. After 2 h, decant the *Acid stage medium*, add the *Buffer stage medium*, and operate the apparatus at the specified conditions. After 45 min, withdraw 10 mL of the solution under test, and pass it through a suitable filter of 0.45-μm pore size.

Analysis: Determine the amount of pantoprazole released to the *Buffer stage medium* using the same chromatographic procedure as directed for the *Acid stage*, with the exception of injecting about 50 μL of the *Standard solution* and *Sample solution*.

Calculate the amount of pantoprazole dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (100/L)$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of pantoprazole sodium in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of pantoprazole, 383.37

M_{r2} = molecular weight of pantoprazole sodium, 405.35

V = volume of *Medium*, 1000 mL

L = Tablet label claim (mg)

Tolerances: NLT 75% (Q) of the labeled amount of pantoprazole is dissolved.

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*. Proceed as directed for *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*, *Method B*, *Procedure*.

Acid stage

Acid stage medium: 0.1 N hydrochloric acid; 1000 mL, degassed

Apparatus 2: 100 rpm, with sinkers

Time: 2 h

Determine the amount of pantoprazole remaining in the Tablet, using the following procedure.

Diluent: Water and acetonitrile (7:3)

Buffer solution: 771 mg/L of ammonium acetate in water. Adjust with acetic acid or ammonium hydroxide to a pH of 8.5 ± 0.1.

Solution A: *Buffer solution* and acetonitrile (7:3)

Solution B: Acetonitrile

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
6	100	0
17	27	73
18	100	0
22	100	0

System suitability solution: Prepare a solution containing 0.0068 mg/mL of USP Pantoprazole Related Compound A in *Diluent*. Transfer 10 mL of this solution to a 100-mL volumetric flask, add 23 mg of USP Pantoprazole Sodium RS, and dilute with *Diluent* to volume.

Acid stage standard solution: 0.23 mg/mL of USP Pantoprazole Sodium RS in *Diluent*

Sample solution: After 2 h in the *Acid stage medium*, carefully remove the Tablet from the vessel and transfer to a suitable volumetric flask. Add 50% of the final volume of *Diluent*, and sonicate for 20 min (but not more than 60 min), swirling the flask every few min. Dilute with *Diluent* to volume to obtain a final concentration of about 0.2 mg/mL of pantoprazole.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 290 nm

Column: 3.9-mm × 15-cm; 5-μm packing L1

Temperature

Column: 30°

Autosampler: 4°

Flow rate: 1 mL/min

Injection size: 20 μL

System suitability

Samples: *System suitability solution* and *Acid stage standard solution*

Suitability requirements

Resolution: NLT 1.5 between pantoprazole related compound A and pantoprazole, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Acid stage standard solution*

Calculate the percentage of pantoprazole released:

$$\text{Result} = A - [(r_U/r_S) \times (C_S/L) \times D_U \times (M_{r1}/M_{r2}) \times 100]$$

A = percentage of pantoprazole as determined in the *Assay*

r_U = peak area from the *Sample solution*

r_S = peak area from the *Acid stage standard solution*

- C_s = concentration of the *Acid stage standard solution* (mg/mL)
 L = Tablet label claim (mg)
 D_u = dilution factor of the *Sample solution*
 M_{r1} = molecular weight of pantoprazole, 383.37
 M_{r2} = molecular weight of pantoprazole sodium, 405.35

Tolerances: NMT 10% (Q) of the labeled amount of pantoprazole is dissolved.

Buffer stage

Buffer stage medium: pH 6.8 phosphate buffer (76.0 g/L of tribasic sodium phosphate dodecahydrate in water. Add 250 mL of this solution to 750 mL of *Acid stage medium*, adjust with hydrochloric acid or sodium hydroxide to a pH of 6.80 ± 0.05); 1000 mL, degassed.

Apparatus 2: 100 rpm, with sinkers

Time: 45 min

Buffer stage standard solution: 1.6 mg/mL of USP Pantoprazole Sodium RS in methanol. This solution is stable for 5 days at room temperature and 7 days when refrigerated. Dilute this solution with *Buffer stage medium* to obtain a concentration of L/1000 mg/mL, where L is the Tablet label claim in mg.

Sample solution: Transfer a Tablet with the sinker to the vessel containing *Acid stage medium*, and proceed as directed for the *Acid stage*. After 2 h, remove the *Acid stage medium*, add the *Buffer stage medium*, and operate the apparatus under the specified conditions. After 45 min, withdraw 10 mL of the solution under test, and pass it through a suitable filter of 0.45- μ m pore size.

Analytical wavelength: UV 289 nm

Path length cell: 1 cm

Blank: *Buffer stage medium*

Calculate the percentage of pantoprazole released:

$$\text{Result} = (A_u/A_s) \times (C_s/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

- A_u = absorbance of the *Sample solution*
 A_s = absorbance of the *Buffer stage standard solution*
 C_s = concentration of the *Buffer stage standard solution* (mg/mL)
 L = Tablet label claim (mg)
 M_{r1} = molecular weight of pantoprazole, 383.37
 M_{r2} = molecular weight of pantoprazole sodium, 405.35
 V = volume of *Buffer stage medium*, 1000 mL

Tolerances: NLT 75% (Q) of the labeled amount of pantoprazole is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

Organic Impurities

PROCEDURE

Mobile phase, System suitability solution, Sample solution, and Chromatographic system: Prepare as directed in the *Assay*.

Standard solution: 0.0004 mg/mL. Dilute the *Standard solution*, prepared as directed in the *Assay*, with 0.02 N sodium hydroxide.

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

[NOTE—Identify the components on the basis of the relative retention times shown in *Impurity Table 1*.]

Resolution: NLT 3 between pantoprazole and pantoprazole related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for pantoprazole, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms for at least three times the retention time of the pantoprazole peak. Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

- r_u = peak response for each impurity from the *Sample solution*
 r_s = peak response from the *Standard solution*
 C_s = concentration of USP Pantoprazole Sodium RS in the *Standard solution* (mg/mL)
 C_u = nominal concentration of pantoprazole in the *Sample solution* (mg/mL)
 M_{r1} = molecular weight of pantoprazole, 383.37
 M_{r2} = molecular weight of pantoprazole sodium, 405.35

Acceptance criteria: The limits are given in *Impurity Table 1*. The reporting level for impurities is 0.1%.

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pantoprazole	1.0	—
Related compounds D ^a and F ^b	1.2	0.5 ^c
Pantoprazole related compound A ^d	1.3	0.5
Pantoprazole related compound B ^e	2.7	0.3
Any other individual impurity	—	0.2
Total impurities	—	1.0

^a 5-(Difluoromethoxy)-2-[(RS)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1-methyl-1H-benzimidazole.

^b 6-(Difluoromethoxy)-2-[(RS)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1-methyl-1H-benzimidazole.

^c Impurities D and F are not fully resolved and should be integrated together.

^d 5-(Difluoromethoxy)-2-[[[(3,4-dimethoxy-2-pyridyl)methyl]sulfonyl]-1H-benzimidazole.

^e 5-(Difluoromethoxy)-2-[[[(3,4-dimethoxy-2-pyridyl)methyl]thio]-1H-benzimidazole.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **LABELING:** Label Tablets to indicate that they must not be split, chewed, or crushed before administration. When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**
 - USP Pantoprazole Related Compound A RS
5-(Difluoromethoxy)-2-[[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfonyl]-1H-benzimidazole.
C₁₆H₁₅F₂N₃O₅S 399.37
 - USP Pantoprazole Related Compound B RS
5-(Difluoromethoxy)-2-[[[(3,4-dimethoxy-2-pyridinyl)methyl]thio]-1H-benzimidazole.
C₁₆H₁₅F₂N₃O₃S 367.37
 - USP Pantoprazole Sodium RS

Papain

Papain [9001-73-4].

DEFINITION

Papain is a purified proteolytic substance derived from *Carica papaya* Linné (Fam. Caricaceae). Papain, prepared as directed in the Assay, contains NLT 6000 Units/mg. Papain of a higher digestive power may be reduced to the official standard by admixture with papain of lower activity, lactose, or other suitable diluents.

One USP Unit of Papain activity is that which releases the equivalent of 1 µg of tyrosine from a specified casein substrate under the conditions of the Assay, using the enzyme concentration that liberates 40 µg of tyrosine per mL of Sample solution.

ASSAY

• CASEIN DIGESTIVE POWER

Dibasic sodium phosphate solution: Dissolve 7.1 g of anhydrous dibasic sodium phosphate in water to make 1 L. Add 1 drop of toluene as a preservative.

Citric acid solution: Dissolve 10.5 g of citric acid monohydrate in water to make 1 L. Add 1 drop of toluene as a preservative.

Casein substrate: Disperse 1 g of Hammersten-type casein in 50 mL of Dibasic sodium phosphate solution. Place in a boiling water bath for 30 min with occasional stirring. Cool to room temperature, and add Citric acid solution to adjust to a pH of 6.0 ± 0.1 . Stir the solution rapidly and continuously during the addition of the Citric acid solution to prevent precipitation of the casein. Dilute with water to 100 mL. Prepare fresh daily.

Buffer solution: Dissolve 3.55 g of anhydrous dibasic sodium phosphate in 400 mL of water in a 500-mL volumetric flask. Add 7.0 g of disodium edetate and 3.05 g of cysteine hydrochloride monohydrate. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 6.0 ± 0.1 , dilute with water to volume, and mix. Prepare fresh daily.

Trichloroacetic acid solution: 300 mg/mL of reagent-grade trichloroacetic acid. This solution may be stored at room temperature.

Standard solution: Weigh accurately 100 mg of USP Papain RS in a 100-mL volumetric flask, and add Buffer solution to dissolve. Dilute with Buffer solution to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with Buffer solution to volume, and mix. Use within 30 min after preparation.

Sample solution: Weigh accurately an amount of Papain equivalent to about 100 mg of USP Papain RS in a 100-mL volumetric flask, and add Buffer solution to dissolve. Dilute with Buffer solution to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with Buffer solution to volume, and mix. Use within 30 min after preparation.

Analysis: Into each of 12 test tubes (18 × 150 mm) pipet 5.0 mL of Casein solution. Place in a water bath at 40°, and allow 10 min to reach bath temperature. The tests are run in duplicate except for the blanks. Into each of two of the tubes labeled S_1 pipet 1.0 mL of the Standard solution and 1.0 mL of Buffer solution. Mix by swirling, note zero time, insert the stopper, and replace in the bath. Into each of two tubes labeled S_2 pipet 1.5 mL of the Standard solution and 0.5 mL of Buffer solution, and proceed as before. Repeat this procedure for two more tubes labeled S_3 to which 2.0 mL of the Standard solution is added, and for two tubes labeled U_2 to which 1.5 mL of the Sample solution and 0.5 mL of Buffer solution are added. After 60 min, accurately timed, add to all 12 tubes 3.0 mL of Trichloroacetic acid solution, and shake vigorously. With the four tubes to which neither the Standard solution nor the Sample solution was added, prepare blanks by pipeting, respec-

tively, 1.0 mL of the Standard solution and 1.0 mL of Buffer solution; 1.5 mL of the Standard solution and 0.5 mL of Buffer solution; 2.0 mL of the Standard solution; and 1.5 mL of the Sample solution and 0.5 mL of Buffer solution. Replace all tubes in the 40° water bath for 30–40 min to allow the precipitated protein to coagulate fully. Pass through filter paper of medium pore size, discarding the first 3 mL of the filtrate (filtrates used are clear). Read the absorbances, at 280 nm, of the filtrates of all solutions against their respective blanks. Plot the absorbance readings for S_1 , S_2 , and S_3 against the enzyme concentration of each corresponding level.

By interpolation from this curve, taking into consideration dilution factors, calculate the potency in Units in the weight of Papain taken:

$$\text{Result} = A \times C \times F$$

A = activity of the USP Papain RS (Units/mg)

C = concentration from the standard curve (mg/mL)

F = $100 \times (50/2) \times (10/1.5)$, dilution factor

Acceptance criteria: NLT 6000 Units/mg

SPECIFIC TESTS

• pH (791)

Sample solution: 1 in 50

Acceptance criteria: 4.8–6.2

• LOSS ON DRYING (731)

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 7.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a cool place.

• **USP REFERENCE STANDARDS (11)**

USP Papain RS

Papain Tablets for Topical Solution

DEFINITION

Papain Tablets for Topical Solution contain NLT 100.0% of the labeled potency.

ASSAY

• PROCEDURE

Dibasic sodium phosphate solution: Dissolve 7.1 g of anhydrous dibasic sodium phosphate in water to make 1 L. Add 1 drop of toluene as a preservative.

Citric acid solution: Dissolve 10.5 g of citric acid monohydrate in water to make 1 L. Add 1 drop of toluene as a preservative.

Casein substrate: Disperse 1 g of Hammersten-type casein in 50 mL of Dibasic sodium phosphate solution.

Place in a boiling water bath for 30 min with occasional stirring. Cool to room temperature, and add Citric acid solution to adjust to a pH of 6.0 ± 0.1 . Stir the solution rapidly and continuously during the addition of the Citric acid solution to prevent precipitation of the casein. Dilute with water to 100 mL. Prepare fresh daily.

Buffer solution: Dissolve 3.55 g of anhydrous dibasic sodium phosphate in 400 mL of water in a 500-mL volumetric flask. Add 7.0 g of disodium edetate and 3.05 g of cysteine hydrochloride monohydrate. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 6.0 ± 0.1 , dilute with water to volume, and mix. Prepare fresh daily.

Trichloroacetic acid solution: 300 mg/mL of reagent-grade trichloroacetic acid. This solution may be stored at room temperature.

Standard solution: Weigh accurately 100 mg of USP Papain RS in a 100-mL volumetric flask, and add *Buffer solution* to dissolve. Dilute with *Buffer solution* to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Use within 30 min after preparation.

Sample solution: Place a counted number of Papain Tablets for Topical Solution, equivalent to about 600,000 USP Units of Papain, in a 100-mL volumetric flask, dissolve in *Buffer solution*, dilute with *Buffer solution* to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with *Buffer solution* to volume, and mix.

Analysis: Into each of 12 test tubes (18 × 150 mm) pipet 5.0 mL of *Casein substrate*. Place in a water bath at 40°, and allow 10 min to reach bath temperature. The tests are run in duplicate except for the blanks. Into each of two of the tubes labeled S₁ pipet 1.0 mL of the *Standard solution* and 1.0 mL of *Buffer solution*. Mix by swirling, note zero time, insert the stopper, and replace in the bath. Into each of two other tubes labeled S₂ pipet 1.5 mL of the *Standard solution* and 0.5 mL of *Buffer solution*, and proceed as before. Repeat this procedure for two tubes labeled S₃ to which 2.0 mL of the *Standard solution* is added, and for two tubes labeled U₂ to which 1.5 mL of the *Sample solution* and 0.5 mL of *Buffer solution* are added. After 60 min, accurately timed, add to all 12 tubes 3.0 mL of *Trichloroacetic acid solution*, and shake vigorously. With the four tubes to which neither the *Standard solution* nor the *Sample solution* was added, prepare blanks by pipeting, respectively, 1.0 mL of the *Standard solution* and 1.0 mL of *Buffer solution*; 1.5 mL of the *Standard solution* and 0.5 mL of *Buffer solution*; 2.0 mL of the *Standard solution*; and 1.5 mL of the *Sample solution* and 0.5 mL of *Buffer solution*. Replace all tubes in the 40° water bath for 30–40 min to allow the precipitated protein to coagulate fully. Pass through filter paper of medium pore size, discarding the first 3 mL of the filtrate (filtrates used are clear). Read the absorbances, at 280 nm, of the filtrates of all solutions against their respective blanks. Plot the absorbance readings for S₁, S₂, and S₃ against the enzyme concentration of each corresponding level.

By interpolation from the standard curve, calculate the potency, in USP Units, of the number of Papain Tablets for Topical Solution taken:

$$\text{Result} = A \times C \times F$$

A = activity of the USP Papain RS (Units/mg)

C = concentration from the standard curve (mg/mL)

F = $100 \times (50/2) \times (10/1.5)$, dilution factor

Acceptance criteria: NLT 100.0% of the labeled potency

PERFORMANCE TESTS

- **DISINTEGRATION** (701): NMT 15 min at 23 ± 2°

SPECIFIC TESTS

- **COMPLETENESS OF SOLUTION** (641)

Sample solution: Prepare a solution of 50 Papain Tablets for Topical Solution in 500.0 mL of water, and allow to stand for 4 h.

Analysis: Pass the *Sample solution* through two superimposed, matched-weight, 47-mm-diameter membrane filters of 0.8-μm pore size, and wash the residue by rinsing the flask at the sides of the holder with water. Dry both filters in a desiccator under vacuum, over phosphorus pentoxide for 6–18 h. Weigh the filters separately, and subtract the weight of the lower filter from that of the upper filter.

Acceptance criteria: NMT 50 mg (1 mg/Tablet) difference in weights

- **MICROBIAL ENUMERATION TESTS** (61) AND **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

- **pH** (791)

Sample solution: 1 Tablet in 10 mL of water

Acceptance criteria: 6.9–8.0

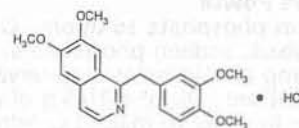
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a cool place.

- **USP REFERENCE STANDARDS** (11)

USP Papain RS

Papaverine Hydrochloride



C₂₀H₂₁NO₄ · HCl 375.85

Isoquinoline, 1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxy-, hydrochloride.

6,7-Dimethoxy-1-veratrylisoquinoline hydrochloride [61-25-6].

» Papaverine Hydrochloride contains not less than 98.5 percent and not more than 100.5 percent of C₂₀H₂₁NO₄ · HCl, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards (11)—

USP Papaverine Hydrochloride RS

Completeness of solution—A 1 in 15 solution in chloroform is clear and free from undissolved solid.

Identification—

A: *Infrared Absorption* (197K).

B: *Ultraviolet Absorption* (197U)—

Solution: 2.5 μg per mL.

Medium: 0.1 N hydrochloric acid.

Absorptivities at 251 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: A solution (1 in 50) responds to the tests for *Chloride* (191).

pH (791): between 3.0 and 4.5, in a solution (1 in 50).

Loss on drying (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.1%.

Limit of cryptopine, thebaine, or other organic impurities—Dissolve 50 mg in 2 mL of sulfuric acid in a small test tube: the resulting solution is not more yellow-brown in color than *Matching Fluid S* (see *Readily Carbonizable Substances Test* (271)), and it is not more pink than a standard prepared, in equal volume, by diluting 3.0 mL of 0.1 N potassium permanganate with water to 1000 mL.

Assay—Dissolve about 700 mg of Papaverine Hydrochloride, accurately weighed, in 80 mL of glacial acetic acid; add 10 mL of mercuric acetate TS and 1 drop of crystal violet TS; and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 37.59 mg of C₂₀H₂₁NO₄ · HCl.

Papaverine Hydrochloride Injection

» Papaverine Hydrochloride Injection is a sterile solution of Papaverine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{20}H_{21}NO_4 \cdot HCl$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

USP Reference standards (11)—

USP Endotoxin RS
USP Papaverine Hydrochloride RS

Identification—

A: Add 2 mL of alcohol to 1 mL of Injection, and evaporate on a steam bath, with the aid of a stream of nitrogen, to dryness. Dry the residue at 105° for 2 hours; it responds to *Identification test A* under *Papaverine Hydrochloride*.

B: It responds to *Identification test C* under *Papaverine Hydrochloride*.

Bacterial Endotoxins Test (85)—It contains not more than 2.9 USP Endotoxin Units per mg of papaverine hydrochloride.

pH (791): not less than 3.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—Transfer 1.0 mL of Injection to a 200-mL volumetric flask, and dilute with water to volume. Pipet 3 mL of this solution into a separator, add 10 mL of water, and render alkaline with 6 N ammonium hydroxide. Extract the alkaloid with successive 5-mL portions of chloroform, and evaporate the extracts to dryness. Dissolve the residue in 0.1 N hydrochloric acid, and dilute with the same medium to 100.0 mL. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Papaverine Hydrochloride RS in 0.1 N hydrochloric acid having a known concentration of about 4.5 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 251 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{20}H_{21}NO_4 \cdot HCl$ in the portion of Injection taken by the formula:

$$6.67C(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Papaverine Hydrochloride RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Papaverine Hydrochloride Tablets

» Papaverine Hydrochloride Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{20}H_{21}NO_4 \cdot HCl$.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Papaverine Hydrochloride RS

Identification—Add a portion of powdered Tablets, equivalent to about 30 mg of papaverine hydrochloride, to 10 mL of 0.1 N hydrochloric acid in a separator. Extract the mixture with 10 mL of chloroform, filter the chloroform phase through paper, evaporate the solvent on a steam bath, and dry the residue at 105° for 2 hours; it responds to *Identification test A* under *Papaverine Hydrochloride*.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{20}H_{21}NO_4 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 250 nm on filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid, in comparison with a Standard solution having a known concentration of USP Papaverine Hydrochloride RS in the same Medium.

Tolerances—Not less than 80% (*Q*) of the labeled amount of $C_{20}H_{21}NO_4 \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Procedure for content uniformity—Transfer 1 finely powdered Tablet to a 250-mL volumetric flask, add 50 mL of water and 3 mL of hydrochloric acid, mix, and allow to stand for 15 minutes with occasional agitation. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate quantitatively and stepwise, if necessary, with water to provide a solution containing approximately 2.4 µg of papaverine hydrochloride per mL. Concomitantly determine the absorbances of this solution and a solution of USP Papaverine Hydrochloride RS, in the same medium at a concentration of about 2.4 µg per mL, in 1-cm cells, at the wavelength of maximum absorbance at about 250 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{20}H_{21}NO_4 \cdot HCl$ in the Tablet by the formula:

$$(TC / D)(A_U / A_S)$$

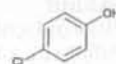
in which *T* is the labeled quantity, in mg, of papaverine hydrochloride in the Tablet; *C* is the concentration, in µg per mL, of USP Papaverine Hydrochloride RS in the Standard solution; *D* is the concentration, in µg per mL, of the solution from the Tablet based upon the labeled quantity per Tablet and the extent of dilution; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 30 mg of papaverine hydrochloride, to a glass-stoppered conical flask, add about 100 mL of 0.1 N hydrochloric acid, and shake by mechanical means for 15 minutes. Filter the mixture into a 200-mL volumetric flask, and add 0.1 N hydrochloric acid to volume. Proceed as directed in the *Assay* under *Papaverine Hydrochloride Injection*, beginning with "Pipet 3 mL of this solution into a separator." Calculate the quantity, in mg, of $C_{20}H_{21}NO_4 \cdot HCl$ in the portion of Tablets taken by the formula:

$$6.67C(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Papaverine Hydrochloride RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Tablets and the Standard solution, respectively.

Parachlorophenol



C_6H_5ClO
Phenol, 4-chloro-;
p-Chlorophenol [106-48-9].

128.56

DEFINITION

Parachlorophenol contains NLT 99.0% and NMT 100.5% of parachlorophenol (C_6H_5ClO).

IDENTIFICATION

- **A.**
Sample solution: 10 mg/mL of Parachlorophenol
Analysis: Add bromine TS dropwise to the *Sample solution*.
Acceptance criteria: A white precipitate is formed; at first it redissolves, but then it becomes permanent as an excess of the reagent is added.
- **B.**
Sample solution: 10 mg/mL of Parachlorophenol
Analysis: Add 1 drop of ferric chloride TS to 10 mL of *Sample solution*.
Acceptance criteria: The solution acquires a violet-blue color.
- **C.**
Analysis: Heat a few crystals, held on a copper wire, in the edge of a nonluminous flame.
Acceptance criteria: A green color is imparted to the flame.
- **D.**
Sample: 1 g of Parachlorophenol
Analysis: Mix the *Sample* and 5 mL of sodium hydroxide solution (1 in 3), then add 1.5 g of monochloroacetic acid. Shake, and heat on a steam bath for 1 h. Cool, dilute with 15 mL of water, and acidify with hydrochloric acid. Extract with 50 mL of ether, wash the ether solution with 10 mL of cold water, then extract the ether solution with 25 mL of sodium carbonate solution (1 in 20). Acidify the solution with hydrochloric acid, collect the resulting precipitate on a filter, and recrystallize it from hot water.
Acceptance criteria: The resulting parachlorophenoxyacetic acid melts between 154° and 158°.

ASSAY• **PROCEDURE**

Sample: 1 g of Parachlorophenol
Titrimetric system
 (See *Titrimetry* (541).)
Mode: Residual titration
Titrant: 0.1 N bromine VS
Back-titrant: 0.1 N sodium thiosulfate VS
Endpoint detection: Visual
Analysis: Transfer the *Sample* to a 500-mL volumetric flask, and dissolve and dilute with water to volume. Transfer a 25.0-mL portion of the solution to an iodine flask, cool in an ice bath to 4°, and add 20.0 mL of *Titrant*. Add 5 mL of hydrochloric acid, and immediately insert the stopper. Maintain the flask at a temperature of 4° for 30 min, shaking at frequent intervals. Allow it to stand for 15 min, remove the stopper just sufficiently to introduce quickly 5 mL of potassium iodide solution (1 in 5), taking care that no bromine vapor escapes, and at once insert the stopper in the flask. Shake thoroughly, remove the stopper, and rinse it and the neck of the flask with a small portion of water, allowing the washings to flow into the flask. Shake the mixture, and titrate the liberated iodine with *Back-titrant*, using 3 mL of starch TS as the indicator. Perform a blank determination. Each mL of 0.1 N bromine is equivalent to 3.214 mg of parachlorophenol (C_6H_5ClO).
Acceptance criteria: 99.0%–100.5%

IMPURITIES• **LIMIT OF NONVOLATILE RESIDUE**

Sample: 1 g of Parachlorophenol
Analysis: Heat the *Sample* in a tared container on a steam bath until it is volatilized, and dry at 105° for 1 h.

Acceptance criteria: NMT 0.1% of residue remains.

• **LIMIT OF CHLORIDE**

Sample solution: 10 mg/mL of Parachlorophenol
Analysis: Acidify 10 mL of *Sample solution* with 2 N nitric acid, and add a few drops of silver nitrate TS.
Acceptance criteria: No turbidity or opalescence is produced.

SPECIFIC TESTS• **CLARITY AND REACTION OF SOLUTION**

Sample solution: 10 mg/mL of Parachlorophenol
Acceptance criteria: Solution is clear and is acid to litmus.

• **CONGEALING TEMPERATURE (651):** Between 42° and 44°**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Camphorated Parachlorophenol**DEFINITION**

Camphorated Parachlorophenol is a triturated mixture that contains NLT 33.0% and NMT 37.0% of parachlorophenol (C_6H_5ClO) and NLT 63.0% and NMT 67.0% of camphor ($C_{10}H_{16}O$). The sum of the percentages of parachlorophenol and camphor is NLT 97.0% and NMT 103.0%.

ASSAY• **PARACHLOROPHENOL**

Sample: 1 g
Analysis: Add the *Sample* to a wide-mouth conical flask. Add a few glass beads, 6 mL of sodium hydroxide solution (1 in 2), and 130 mL of water. Heat the solution to boiling, add 70 mL of potassium permanganate solution (3 in 50), and continue to boil for 20 min. To the hot solution add 40 mL of 0.1 N silver nitrate. Add 50 mL of 18 N sulfuric acid, and sodium sulfite crystals, in divided portions and with swirling until the permanganate color is discharged and no manganese dioxide remains. Boil until the vapors are no longer acid to litmus, keeping the volume nearly constant by the addition of water. Add 5 mL of nitric acid, and continue to boil for 5 min. Cool, and collect the precipitate on a tared filtering crucible, wash well with water, then with 10 mL of alcohol. Dry at 105° for 1 h, cool, and weigh. Each 1.000 g of the silver chloride so obtained is equivalent to 897.0 mg of parachlorophenol (C_6H_5ClO).

Acceptance criteria

Parachlorophenol: 33.0%–37.0% of parachlorophenol (C_6H_5ClO)

Total: The sum of the percentages of parachlorophenol and camphor is 97.0%–103.0%.

• **CAMPBOR**

Sample solution: Transfer about 300 mg of Camphorated Parachlorophenol to a 200-mL pressure bottle containing 50 mL of freshly prepared dinitrophenylhydrazine TS.

Analysis: Close the pressure bottle, immerse it in a water bath, and maintain it at about 75° for 4 h. Cool to room temperature, then transfer the contents to a beaker with the aid of 100 mL of 3 N sulfuric acid and allow it to stand overnight. Collect the precipitate on a tared filtering crucible, wash with 100 mL of 3 N sulfuric acid and then with 75 mL of cold water, in divided portions, to remove the acid. Dry at 80° for 2 h, cool, and weigh. The weight of the precipitate so obtained, multiplied by 0.4581, represents the weight of camphor ($C_{10}H_{16}O$) in the sample taken.

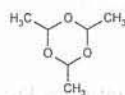
Acceptance criteria

Camphor: 63.0%–67.0% of camphor ($C_{10}H_{16}O$)

Total: The sum of the percentages of parachlorophenol and camphor is 97.0%–103.0%.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Paraldehyde

$C_6H_{12}O_3$ 132.16

1,3,5-Trioxane, 2,4,6-trimethyl-
2,4,6-Trimethyl-s-trioxane [123-63-7].

» **NOTE**—Paraldehyde is subject to oxidation to form acetic acid. It may contain a suitable stabilizer.

Packaging and storage—Preserve in well-filled, tight, light-resistant containers, preferably of Type I or Type II glass, holding not more than 30 mL, at a temperature not exceeding 25°. Paraldehyde may be shipped in bulk containers holding a minimum of 22.5 kg (50 lb) to commercial drug repackagers only.

Labeling—The label of all containers of Paraldehyde, including those dispensed by the pharmacist, includes a statement directing the user to discard the unused contents of any container that has been opened for more than 24 hours.

NOTE—The label of bulk containers of Paraldehyde directs the commercial drug repackager to demonstrate compliance with the USP purity tests for Paraldehyde immediately prior to repackaging, and not to repackage from a container that has been opened longer than 24 hours.

Identification—Heat it with a small quantity of 2 N sulfuric acid: acetaldehyde, recognizable by its pungent odor, is produced.

Congeeing temperature (651): not lower than 11°.

Distilling range, Method I (721)—It distills completely between 120° and 126°, a correction factor of 0.050° per mm being applied as necessary.

Acidity—To a solution of 6.0 mL in 100 mL of water add 5 drops of phenolphthalein TS, and titrate with 1.0 N sodium hydroxide: not more than 0.50 mL is required to produce a pink color (0.5% as acetic acid).

Chloride—To 5 mL of a solution (1 in 10) add 1 drop of nitric acid and 3 drops of silver nitrate TS: no opalescence is produced immediately.

Sulfate—To 5 mL of a solution (1 in 10) add 1 drop of hydrochloric acid and 3 drops of barium chloride TS: no turbidity is produced.

Limit of nonvolatile residue—Heat 5.0 mL in a small, tared evaporating dish on a steam bath: no disagreeable odor is noticeable as the last portions evaporate, and, when dried at 105° for 1 hour, not more than 3 mg of residue remains (0.06%).

Limit of acetaldehyde—Place 100 mL of water in a 250-mL conical flask, add 5.0 mL of Paraldehyde, and shake the mixture gently until solution is complete. Add 5 mL of hydroxylamine hydrochloride solution (3.5 in 100). Shake the mixture gently for 30 seconds, add methyl orange TS, and titrate immediately with 0.50 N sodium hydroxide. Per-

form a blank titration: the difference between the titers does not exceed 1 mL of 0.50 N sodium hydroxide (0.4%).

Paregoric**DEFINITION**

Paregoric contains NLT 35 mg and NMT 45 mg of anhydrous morphine in each 100 mL.

Prepare Paregoric as follows.

Powdered Opium	4.3 g
Suitable Essential Oil(s)	—
Benzoic Acid	3.8 g
Diluted Alcohol	900 mL
Glycerin	38 mL
To make	950 mL

Prepare a diluted alcohol mixture containing 400 mg of *Benzoic Acid*, 4 mL of *Glycerin*, and sufficient *Suitable Essential Oil(s)* in each 100 mL of *Diluted Alcohol*. Separately, macerate the *Powdered Opium*, *Benzoic Acid*, and *Suitable Essential Oil(s)* for 5 days, with occasional agitation, in a mixture of *Diluted Alcohol* and *Glycerin*. Then filter, and pass enough *Diluted Alcohol* through the filter to obtain 950 mL of total filtrate. Assay a portion of this filtrate as directed herein, and dilute the remainder with a sufficient quantity of the previously prepared diluted alcohol mixture to yield a solution containing 40 mg of anhydrous morphine in each 100 mL.

Paregoric may also be prepared by using *Opium* or *Opium Tincture* instead of *Powdered Opium*, the anhydrous morphine content being adjusted to 40 mg in each 100 mL and the alcohol content being adjusted to 45%.

ASSAY**• PROCEDURE**

Mobile phase A: 1-in-5 solution of triethylamine in water-saturated chloroform

Mobile phase B: 1-in-100 solution of triethylamine in water-saturated chloroform

Chromatographic tubes: Prepare three similar tubes, each about 260 mm long and consisting of about 200 mm of 25-mm tubing and about 6 cm of 6-mm tubing. In each of the tubes, place a pledget of glass wool at a point where the 6-mm tubing is constricted slightly, about 2 cm from the junction.

Citrate buffer: 0.1 M sodium citrate and 0.1 M citric acid (50:50)

Standard solution: Dissolve a quantity of USP Morphine Sulfate RS equivalent to about 40 mg of anhydrous morphine in 0.5 mL of triethylamine contained in a 100-mL volumetric flask, and add methanol to volume. Pipet 10 mL of this solution into a 50-mL volumetric flask, add 1 mL of triethylamine and 1 mL of hydrochloric acid, and add water-saturated chloroform to volume.

Sample solution: Evaporate 10.0 mL of Paregoric (equivalent to about 4 mg of morphine) on a steam bath under a stream of air to about 2 mL, and cool. [NOTE—Avoid reducing the volume to less than 2 mL.] Add 0.5 mL of *Citrate buffer*.

Chromatographic columns: Fill the three tubes with adsorbent prepared as follows, using chromatographic siliceous earth as the base of the adsorbent, and tamp it firmly in place. After filling, place a small pad of glass wool above each column packing.

Column 1: Pack in two layers, the lower layer consisting of 3 g of chromatographic siliceous earth mixed with 2 mL of *Citrate buffer* and the upper layer of 3 g of chromatographic siliceous earth mixed with the *Sample solution*. Dry-rinse the beaker in which the

components of the two layers have been mixed with 1 g of chromatographic siliceous earth, and add it also to the top of the column.

Column II: Pack with 3 g of chromatographic siliceous earth mixed with 2 mL of dibasic potassium phosphate solution (1 in 5.75).

Column III: Pack with 3 g of chromatographic siliceous earth mixed with 2 mL of sodium hydroxide solution (1 in 50).

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 255–360 nm

Cell: 1 cm

Blank: Chloroform

Analysis

[NOTE—(1) Use water-saturated solvents throughout this procedure; (2) prepare eluants fresh daily; and (3) avoid bringing the solutions into contact with metal.]

Wash *Column I* with 100 mL of ether, followed by 100 mL of chloroform. Rinse the tip of the column with chloroform, and discard the solvents. In the following operations, rinse each column tip before discarding the column or changing receivers.

Mount the three columns vertically so that the effluent from *Column I* flows into *Column II*, and the effluent from the latter flows into *Column III*. Pass through the three columns 5 mL of *Mobile phase A*, followed by four 10-mL portions of *Mobile phase B*, allowing each portion to pass through completely before subsequent additions. Discard *Column I*.

Pass three 5-mL portions of *Mobile phase B* through the two remaining columns. Discard *Column II*.

Wash *Column III* successively with 10 mL of *Mobile phase B*, 50 mL of chloroform, 2 mL of a 1-in-10 solution of glacial acetic acid in chloroform, and 50 mL of a 1-in-100 solution of glacial acetic acid in chloroform. Discard all washings. Arrange to collect eluate from *Column III* in a 50-mL volumetric flask containing 10 mL of methanol and 1 mL of hydrochloric acid. Elute the column with 5 mL of *Mobile phase A*, followed by 33 mL of *Mobile phase B*. Dilute with chloroform to volume, and mix.

Concomitantly record the spectra of this solution and the *Standard solution*, and plot the corresponding wavelength-absorbance curves. Correct the absorbance of each solution, at the wavelength of maximum absorbance at about 285 nm, by extrapolating the portion of the base-line curve between 340 and 310 nm to this wavelength.

Calculate the weight of anhydrous morphine, in mg, in 100 mL of Paregoric taken:

$$\text{Result} = (A_U/A_S) \times W \times F$$

A_U = corrected absorbance of the *Sample solution*

A_S = corrected absorbance of the *Standard solution*

W = weight of anhydrous morphine in the 50 mL of *Standard solution* (mg)

F = dilution factor, 10

Acceptance criteria: 35–45 mg of anhydrous morphine per 100 mL of Paregoric

OTHER COMPONENTS

- **ALCOHOL DETERMINATION, Method II (611):** 43.0%–47.0% of C_2H_5OH , using acetone as the internal standard

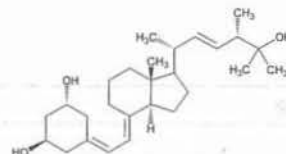
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

• USP REFERENCE STANDARDS (11)

USP Morphine Sulfate RS

Paricalcitol



$C_{27}H_{44}O_3$

416.64

19-Nor-1- α ,25-dihydroxyvitamin D_2 ;

(1 α ,3 β ,7 E ,22 E)-19-Nor-9,10-secoergosta-5,7,22-triene-1,3,25-triol;

(7 E ,22 E)-19-Nor-9,10-secoergosta-5,7,22-triene-1 α ,3 β ,25-triol [131918-61-1].

DEFINITION

Paricalcitol contains NLT 97.0% and NMT 103.0% of paricalcitol ($C_{27}H_{44}O_3$), calculated on the dried basis.

[CAUTION—Handle Paricalcitol with exceptional care because it is very potent. Care should be taken to prevent inhaling particles of Paricalcitol and exposing the skin to it.]

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

[NOTE—Protect paricalcitol solutions from light.]

Mobile phase: Methanol and water (4:1)

Diluent: Methanol and water (1:1)

Standard solution: Dilute USP Paricalcitol Solution RS with *Diluent* to obtain a solution containing 5.0 $\mu\text{g/mL}$ of paricalcitol.

Sample solution: Transfer an accurately weighed amount of Paricalcitol to a suitable volumetric flask, add dehydrated alcohol (approximately 1 mL of dehydrated alcohol per each 0.5 mg of paricalcitol), sonicate to dissolve, and dilute with *Diluent* to volume. Further dilute this solution with *Diluent* to obtain a solution containing 5.0 $\mu\text{g/mL}$ of paricalcitol.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L1

Flow rate: 2 mL/min

Injection volume: 100 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of paricalcitol ($C_{27}H_{44}O_3$) in the portion of Paricalcitol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of paricalcitol in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Paricalcitol in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

• ORGANIC IMPURITIES

[NOTE—Unless otherwise specified, protect paricalcitol solutions from light.]

Diluent: Dehydrated alcohol and water (50:50)

Solution A: Acetonitrile and water (5:95)

Solution B: Acetonitrile and methanol (75:25)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	42	58
11 ^a	42	58
20	0	100
27	0	100
27.1	42	58
30	42	58

^a Determine the retention time of the paricalcitol peak using the *Standard solution*. Adjust the start of the gradient to be 1.0 ± 0.1 min prior to the retention time of paricalcitol and accordingly adjust the remaining gradient times.

System suitability stock solution: Prepare a 50- $\mu\text{g/mL}$ solution of paricalcitol from USP Paricalcitol Solution RS in dehydrated alcohol. Using a colorless glass container, expose the solution to ultraviolet light at 254 nm. Paricalcitol undergoes partial degradation to 7Z-paricalcitol. A degradation of at least 0.2% of paricalcitol to 7Z-paricalcitol [(7Z,22E)-19-nor-9,10-secoergosta-5,7,22-triene-1 α ,3 β ,25-triol] must be obtained, based on the corresponding peaks. If it is not obtained, expose the solution to ultraviolet light again.

System suitability solution: *System suitability stock solution* and water (1:1)

Standard stock solution: 5 $\mu\text{g/mL}$ of paricalcitol from USP Paricalcitol Solution RS in *Diluent*

Standard solution: 0.15 $\mu\text{g/mL}$ of paricalcitol from *Standard stock solution* in *Diluent*

Sensitivity solution: 0.05 $\mu\text{g/mL}$ of paricalcitol from *Standard solution* in *Diluent*

Sample stock solution: 200 $\mu\text{g/mL}$ of Paricalcitol in dehydrated alcohol

Sample solution: 100 $\mu\text{g/mL}$ of Paricalcitol from *Sample stock solution* in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm \times 10-cm; 2.7- μm packing L1

Column temperature: 30°

Flow rate: 0.9 mL/min

Injection volume: 25 μL

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—The relative retention times for paricalcitol and 7Z-paricalcitol are 1.0 and 1.06, respectively.]

Suitability requirements

Resolution: NLT 1.5 between paricalcitol and 7Z-paricalcitol, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Paricalcitol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of paricalcitol from the *Standard solution*

C_S = concentration of paricalcitol in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of Paricalcitol in the *Sample solution* ($\mu\text{g/mL}$)

F = relative response factor (see Table 2)

Acceptance criteria: See Table 2. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Paricalcitol	1.0	—	—
22Z-Paricalcitol ^a	1.23	0.64	0.15
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	0.5

^a (7E,22Z)-19-Nor-9,10-secoergosta-5,7,22-triene-1 α ,3 β ,25-triol.

SPECIFIC TESTS

• LOSS ON DRYING

(See *Thermal Analysis* (891).)

Sample: 8 mg of Paricalcitol

Analysis: Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument. Heat at a rate of 5°/min between ambient temperature and 150° in an atmosphere of nitrogen at a flow rate of 40 mL/min. Determine the accumulated loss in weight from the thermogram.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store under argon in a freezer.

• **USP REFERENCE STANDARDS (11)**

USP Paricalcitol RS

USP Paricalcitol Solution RS

Paricalcitol Injection

DEFINITION

Paricalcitol Injection is a sterile solution of Paricalcitol in a mixture of Water for Injection, Propylene Glycol, and Alcohol, or other suitable solvents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of paricalcitol ($\text{C}_{27}\text{H}_{44}\text{O}_3$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

[NOTE—Protect paricalcitol solutions from light.]

Mobile phase: Methanol and water (4:1)

Diluent: Methanol and water (1:1)

Standard solution: Dilute USP Paricalcitol Solution RS with *Diluent* to obtain a solution having a concentration of paricalcitol similar to that of the Injection.

Sample solution: Use the Injection.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 2 mL/min

Injection volume: 100–200 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of paricalcitol ($C_{27}H_{44}O_3$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of paricalcitol in the *Standard solution*, calculated on the basis of the content of paricalcitol in the USP Paricalcitol Solution RS (μg/mL)

C_U = nominal concentration of paricalcitol in the *Sample solution* (μg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS**• CONTENT OF PROPYLENE GLYCOL AND ALCOHOL (if present)**

Mobile phase: 0.01 N sulfuric acid solution, filtered and degassed

Alcohol stock solution: Transfer 2.0 mL of dehydrated alcohol to a 10-mL volumetric flask, and dilute with water to volume.

Propylene glycol stock solution: Transfer 3.0 mL of propylene glycol to a 10-mL volumetric flask, and dilute with water to volume.

Standard solution: Transfer 5.0 mL each of *Alcohol stock solution* and *Propylene glycol stock solution* to a 50-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 5.0 mL of Injection to a 50-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 7.8-mm × 30-cm; packing L17

Column temperature: 60°

Flow rate: 0.8 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

[NOTE—Elution order is propylene glycol followed by alcohol.]

Suitability requirements

Resolution: NLT 4.0 between propylene glycol and alcohol

Relative standard deviation: NMT 2.0% for each peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of propylene glycol and alcohol in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of alcohol or propylene glycol from the *Sample solution*

r_S = peak response of alcohol or propylene glycol from the *Standard solution*

C_S = concentration of alcohol or propylene glycol in the *Alcohol stock solution* or *Propylene glycol stock solution* (% v/v)

C_U = nominal concentration of alcohol or propylene glycol (if present) in the Injection (% v/v)

Acceptance criteria

Alcohol: 80.0%–120.0%

Propylene glycol: 80.0%–120.0%

IMPURITIES**• ALUMINUM (206)**

Diluent: Dilute 4 mL of nitric acid with water to 2000 mL.

Matrix modifier: 1.5 mg/mL of magnesium nitrate

Standard stock solution: Proceed as directed for *Standard preparations* in the chapter, beginning with "Treat some aluminum wire" and ending with "Cool, and transfer the solution, with the aid of water, to a 100-mL volumetric flask, and dilute with water to volume". Transfer 2 mL of this solution to a second 100-mL volumetric flask, and dilute with water to volume. Transfer 2 mL of this solution to a third 100-mL volumetric flask, and dilute with water to volume. This solution contains 0.4 μg/mL of aluminum.

Standard solution A: 2.5 ng/mL of aluminum in *Diluent*, from the *Standard stock solution*

Standard solution B: 5.0 ng/mL of aluminum in *Diluent*, from the *Standard stock solution*

Standard solution C: 10 ng/mL of aluminum in *Diluent*, from the *Standard stock solution*

Standard solution D: 20 ng/mL of aluminum in *Diluent*, from the *Standard stock solution*

Standard solution E: 50 ng/mL of aluminum in *Diluent*, from the *Standard stock solution*

Sample solution: Dilute 4.0 mL of Injection with 6.0 mL of *Diluent*, or use an appropriate dilution to obtain a solution having a concentration of NMT 0.02 μg/mL of aluminum.

System suitability solution: Dilute 9.5 mL of the *Sample solution* with 0.5 mL of the *Standard stock solution*. If the resulting solution contains more than 0.04 μg/mL of aluminum, prepare an alternate dilution having a concentration of about 0.02–0.04 μg/mL of aluminum.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry; instrument equipped with a flameless, electrically heated furnace

Lamp: Aluminum hollow-cathode

Analytical wavelength: Aluminum emission line at 309.3 nm

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C*, *Standard solution D*, *Standard solution E*, *Sample solution*, and *System suitability solution*
Under typical conditions, the sample volume is 20 μL, the volume of the *Matrix modifier* is 5 μL, the injection temperature is 100°, and the oven conditions are as listed in *Table 1*. [NOTE—These conditions may be optimized for each instrument.]

Table 1

Step	Temperature
Drying 1	110°
Drying 2	130°
Drying 3	200°
Pyrrolysis	1100°
Read	2300°
Clean out	2450°

Determine the absorbances of the samples. Plot the absorbances of the *Standard solutions* versus the content of aluminum, in ng/mL, drawing a straight line best fitting the five points. The correlation coefficient is NLT 0.995, the recovery for the *System suitability solution* is 80%–120%, and the duplicate injections must agree within 0.0024 µg/mL. From the graph so obtained, determine the quantity of aluminum, C, in µg, found in each mL of the *Sample solution*. Calculate the quantity, in µg/mL, of aluminum in the portion of Injection taken:

$$\text{Result} = C \times D$$

C = measured concentration of aluminum in the *Sample solution* (µg/mL)

D = dilution factor used to prepare the *Sample solution*

Acceptance criteria: NMT 0.5 µg/mL

• ORGANIC IMPURITIES

Diluent: Acetonitrile and water (1:1)

Solution A: Acetonitrile and water (15:85)

Solution B: Acetonitrile

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	65	35
25	5	95
45	5	95

Return to the original conditions, and re-equilibrate the system.

Standard solution: Dilute USP Paricalcitol Solution RS with *Diluent* to obtain a solution having a concentration of paricalcitol equal to 0.5% of the labeled concentration of the Injection.

Degradation stock solution: Dilute 1 mL of USP Paricalcitol Solution RS with *Diluent* to 5 mL.

Degradation solution A: Transfer 1 mL of the *Degradation stock solution* and 0.1 mL of 30% hydrogen peroxide into a 10-mL container, and allow to stand at room temperature for 1 h. Dilute with *Diluent* to 10 mL, and mix.

Degradation solution B: Mix 1 mL of the *Degradation stock solution* and 1 mL of 0.1 N hydrochloric acid, and heat at 70° for 1 h. Cool to room temperature, dilute with *Diluent* to 10 mL, and mix.

Sample solution: Use the Injection.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 252 nm

Columns

Guard: 4.6-mm × 7.5-mm; packing L1

Analytical: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 100–200 µL

System suitability

Samples: *Standard solution* and *Degradation solution B*

Suitability requirements

Resolution: NLT 1.0 between the paricalcitol peak and the related compound D peak, *Degradation solution B*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Diluent*, *Degradation solution A*, *Degradation solution B*, *Standard solution*, and *Sample solution*

Identify the impurities in the *Sample solution* on the basis of the relative retention times of the components of *Degradation solution A* and *Degradation solution B* in Table 3.

Table 3

Name ^a	Degradation Solution	Relative Retention Time	Acceptance Criteria, NMT (%)
Related compound A	A	0.63	1.0
Related compound B	A	0.79	1.0
Related compound C	B	0.89	1.0
Related compound D	B	0.95	1.0
Related compound E ^b	B	1.32	1.0
Related compound F	B	1.57	1.0
Related compound G	B	1.66	1.0
Related compound H	B	1.74	1.0
Related compound I	B	1.79	1.0
Total impurities	—	—	2.0

^a Related compounds A–I are specified unidentified degradation products. No information is available about chemical structures or chemical names for these impurities.

^b This peak is very small; the signal-to-noise ratio is approximately 3–5.

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_u/r_s) \times C_s \times (100/L)$$

r_u = peak response of each impurity from the *Sample solution*

r_s = peak response of paricalcitol from the *Standard solution*

C_s = concentration of paricalcitol in the *Standard solution*, calculated on the basis of the content of paricalcitol in the USP Paricalcitol Solution RS (µg/mL)

L = labeled amount of paricalcitol in the Injection (µg/mL)

Acceptance criteria: See Table 3. Disregard any peak observed in the *Diluent*.

SPECIFIC TESTS

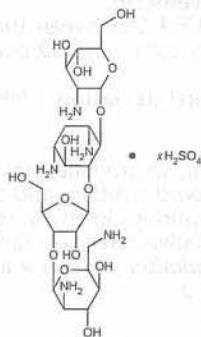
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 10 USP Endotoxin Units/µg of paricalcitol.
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multi-dose containers, preferably of Type I glass. Store at controlled room temperature.

- **USP REFERENCE STANDARDS** (11)
USP Endotoxin RS
USP Paricalcitol Solution RS

Paromomycin Sulfate



$C_{23}H_{45}N_5O_{14} \cdot xH_2SO_4$

D-Streptamine, [O-2-amino-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-O-[O-2,6-diamino-2,6-dideoxy- β -L-idopyranosyl-(1 \rightarrow 3)- β -D-ribofuranosyl-(1 \rightarrow 5)]-2-deoxy-, sulfate (salt).
O-2,6-Diamino-2,6-dideoxy- β -L-idopyranosyl-(1 \rightarrow 3)-O- β -D-ribofuranosyl-(1 \rightarrow 5)-O-[2-amino-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)]-2-deoxystreptamine sulfate (salt) [1263-89-4].

Base 615.63 [59-04-1; 7542-37-2].

» Paromomycin Sulfate is the sulfate salt of an antibiotic substance or substances produced by the growth of *Streptomyces rimosus* var. *paromomycinus*, or a mixture of two or more such salts. It has a potency equivalent to not less than 675 μ g of paromomycin ($C_{23}H_{45}N_5O_{14}$) per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Paromomycin Sulfate RS

Identification—

A: Prepare a test solution in water containing 10 mg of paromomycin per mL. Apply 25 μ L of this solution and 25 μ L of a Standard solution of USP Paromomycin Sulfate RS containing 10 mg of paromomycin per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, place the plate in a developing chamber, and develop the chromatogram in a solvent system consisting of a mixture of freshly prepared ammonium acetate solution (4 in 100), *n*-propyl alcohol, and ammonium hydroxide (30:10:6) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow it to air-dry for 10 minutes. Heat the plate at 105° for 1 hour, allow to cool, and spray with a solution of ninhydrin in butanol (1 in 100). Heat the plate at 105° for 5 minutes: paromomycin appears as a red spot, and the R_f value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

B: It meets the requirements of the tests for *Sulfate* (191).

Specific rotation (781S): between +50° and +55°.

Test solution: 50 mg per mL, in water.

pH (791): between 5.0 and 7.5, in a solution (3 in 100).

Loss on drying (731)—Dry about 100 mg in a capillary-stoppered bottle in vacuum at a pressure not exceeding

5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.

Residue on ignition (281): not more than 2.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

Assay—Proceed with Paromomycin Sulfate as directed under *Antibiotics—Microbial Assays* (81).

Paromomycin Sulfate Capsules

» Paromomycin Sulfate Capsules contain the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of paromomycin ($C_{23}H_{45}N_5O_{14}$).

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Paromomycin Sulfate RS

Identification—Mix the contents of 1 Capsule with water to obtain a solution containing the equivalent of about 20 mg of paromomycin per mL. This test solution responds to the *Identification* test under *Paromomycin Sulfate*.

Disintegration (701): 15 minutes, the use of disks being omitted.

Uniformity of dosage units (905): meet the requirements.

Loss on drying (731)—Dry about 100 mg in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 7.0% of its weight.

Change to read:

Assay—Proceed with Capsules as directed under *Antibiotics—Microbial Assays* (81), blending not less than 5 Capsules for 5 minutes in a high-speed blender with sufficient *Buffer B.3* (CN 1-May-2017) to obtain a stock solution of convenient concentration. Dilute this stock solution quantitatively with the same buffer to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Paromomycin Oral Solution

» Paromomycin Oral Solution contains an amount of Paromomycin Sulfate equivalent to not less than 90.0 percent and not more than 130.0 percent of the labeled amount of paromomycin ($C_{23}H_{45}N_5O_{14}$). It may contain one or more suitable buffers, colors, flavors, preservatives, and solvents.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Paromomycin Sulfate RS

Uniformity of dosage units (905)—

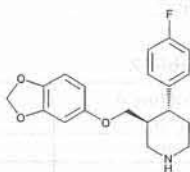
FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698): meets the requirements.
pH (791): between 7.5 and 8.5.

Change to read:

Assay—Proceed with Oral Solution as directed under *Antibiotics—Microbial Assays* (81), diluting an accurately measured volume of Oral Solution with **Buffer B.3** (CN 1-May-2017) to yield a stock solution of convenient concentration. Quantitatively dilute this stock solution with the same buffer to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Paroxetine Hydrochloride



$C_{19}H_{20}FNO_3 \cdot HCl$ 365.83
 Hemihydrate 374.83
 Piperidine, 3-[(1,3-benzodioxol-5-yl)oxy)methyl]-4-(4-fluorophenyl)-, hydrochloride, (3*S*-trans)-; (-)-(3*S*,4*R*)-4-(*p*-Fluorophenyl)-3-[(3,4-methylenedioxy)phenoxy)methyl]piperidine hydrochloride [78246-49-8].

DEFINITION

Paroxetine Hydrochloride is anhydrous or contains one-half molecule of water of hydration. It contains NLT 98.5% and NMT 102.0% of paroxetine hydrochloride ($C_{19}H_{20}FNO_3 \cdot HCl$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- A. INFRARED ABSORPTION** (197M)
Standard: Dissolve USP Paroxetine Hydrochloride RS in a mixture of water and isopropyl alcohol (1 in 10). Heat to 70° to dissolve, recrystallize, and dry the residue under vacuum at 50° for 3 h.
Sample: Dissolve Paroxetine Hydrochloride in a mixture of water and isopropyl alcohol (1 in 10). Heat to 70° to dissolve, recrystallize, and dry the residue under vacuum at 50° for 3 h.
Acceptance criteria: Meets the requirements
- B. IDENTIFICATION TESTS—GENERAL, Chloride** (191)
Sample solution: 10 mg/mL of Paroxetine Hydrochloride in methanol and water (1:1)
Acceptance criteria: Meets the requirements

ASSAY

- PROCEDURE**
Buffer: 0.05 M ammonium acetate in water. Adjust with glacial acetic acid to a pH of 4.5.
Mobile phase: Acetonitrile, *Buffer*, and triethylamine (30:70:1). [NOTE—The ratio for acetonitrile, *Buffer*, and triethylamine may be varied between 25:75:1 and 40:70:1 to meet system suitability requirements.] Adjust with glacial acetic acid to a pH of 5.5.
System suitability solution: 0.5 mg/mL each of USP Paroxetine Hydrochloride RS and USP Paroxetine Related Compound B RS
Standard solution: 0.5 mg/mL of USP Paroxetine Hydrochloride RS
Sample solution: 0.5 mg/mL of Paroxetine Hydrochloride

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 295 nm

Column: 4.6-mm × 25-cm; packing L13

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The approximate relative retention times for paroxetine related compound B and paroxetine are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between paroxetine related compound B and paroxetine

Tailing factor: NMT 2.0 for paroxetine

Relative standard deviation: NMT 2.0% for paroxetine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of paroxetine hydrochloride ($C_{19}H_{20}FNO_3 \cdot HCl$) in the portion of Paroxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

- RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-Jan-2018)

LIMIT OF PAROXETINE RELATED COMPOUND C

Mobile phase: *n*-Hexane, absolute alcohol, trifluoroacetic acid, and water (900:100:2:2)

Diluent: *n*-Hexane and absolute alcohol (1:1)

System suitability solution: 0.1 mg/mL each of Paroxetine Hydrochloride and USP Paroxetine Related Compound C RS in *Diluent*

Standard solution: 0.1 mg/mL of USP Paroxetine Related Compound C RS in *Diluent*

Sample solution: 5 mg/mL of Paroxetine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 295 nm

Column: 4.6-mm × 25-cm; packing L51

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for paroxetine related compound C and paroxetine are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between paroxetine and paroxetine related compound C, *System suitability solution*

Tailing factor: NMT 2.5 for the paroxetine related compound C peak, *System suitability solution*

Relative standard deviation: NMT 10.0% for paroxetine related compound C, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of paroxetine related compound C in the portion of Paroxetine Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*
 r_s = peak response from the *Standard solution*
 C_s = concentration of paroxetine related compound C in the *Standard solution* (mg/mL)
 C_u = concentration of Paroxetine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.1% of paroxetine related compound C

• **LIMIT OF 1-METHYL-4-(p-FLUOROPHENYL)-1,2,3,6-TETRAHYDROPYRIDINE**

Solution A: Dissolve 30 g of sodium perchlorate in 900 mL of water. Add 3.5 mL of phosphoric acid and 2.4 mL of triethylamine. Dilute with water to 1000 mL. Adjust with phosphoric acid or triethylamine to a pH of 2.0.

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
20	80	20
27	55	45
36	55	45
38	85	15
45	85	15

Diluent: Acetonitrile and water (1:4)

Standard solution: 42 ng/mL of 1-methyl-4-(p-fluorophenyl)-1,2,3,6-tetrahydropyridine, obtained from USP Paroxetine Related Compound E Mixture RS, in *Diluent*

Sample solution: 42 mg/mL of Paroxetine Hydrochloride in *Diluent*. Sonicate as needed to aid dissolution.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 242 nm

Column: 4.0-mm × 25-cm; packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 75 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for 1-methyl-4-(p-fluorophenyl)-1,2,3,6-tetrahydropyridine and paroxetine are about 0.6 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 15.0% for 1-methyl-4-(p-fluorophenyl)-1,2,3,6-tetrahydropyridine, determined from three replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 1-methyl-4-(p-fluorophenyl)-1,2,3,6-tetrahydropyridine in the portion of Paroxetine Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times I \times 100$$

r_u = peak response from the *Sample solution*
 r_s = peak response from the *Standard solution*

C_s = concentration of USP Paroxetine Related Compound E Mixture RS in the *Standard solution* (mg/mL)

C_u = concentration of paroxetine in the *Sample solution* (mg/mL)

I = fraction of 1-methyl-4-(p-fluorophenyl)-1,2,3,6-tetrahydropyridine in the USP Paroxetine Related Compound E Mixture RS

Acceptance criteria: NMT 0.0001%

• **ORGANIC IMPURITIES, PROCEDURE 1**

Perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*, depending on the synthetic route. *Organic Impurities, Procedure 2* is recommended if paroxetine related compound F or G are potential impurities.

Solution A: Tetrahydrofuran, water, and trifluoroacetic acid (20:180:1)

Solution B: Acetonitrile, tetrahydrofuran, and trifluoroacetic acid (180:20:1)

Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
30	80	20
50	20	80
60	20	80
70	80	20

Diluent: Tetrahydrofuran and water (1:9)

System suitability solution: 1 mg/mL of USP Paroxetine System Suitability Mixture A RS in *Diluent*. Sonication may be necessary to achieve complete dissolution.

Standard solution: 0.001 mg/mL of USP Paroxetine Hydrochloride RS in *Diluent*

Sample solution: 1 mg/mL of Paroxetine Hydrochloride in *Diluent*. Sonicate to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—Identify the peaks using the relative retention times given in Table 3.]

Suitability requirements

Resolution: NLT 2.0 between paroxetine related compound A and paroxetine related compound B

Tailing factor: 0.8–2.0 for paroxetine related compound A

Relative standard deviation: NMT 2.0% for paroxetine related compound A

Analysis

Samples: *Diluent*, *Standard solution*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Paroxetine Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak area of each impurity from the *Sample solution*, excluding peaks obtained from the chromatogram of the *Diluent*

r_s = peak area of paroxetine from the *Standard solution*

C_s = concentration of USP Paroxetine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Paroxetine Hydrochloride, on the anhydrous basis, in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Paroxetine related compound A	0.66	0.1
Paroxetine related compound B	0.73	0.3
Paroxetine	1.0	—
Any unspecified impurity	—	0.1
Total impurities	—	1.0

• ORGANIC IMPURITIES, PROCEDURE 2

Buffer: Dissolve 3.4 g of monobasic potassium phosphate and 3.4 g of tetrabutylammonium hydrogen sulfate in 1.0 L of water.

Solution A: Acetonitrile and *Buffer* (2:98)

Solution B: Acetonitrile and *Buffer* (40:60)

Mobile phase: See Table 4.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
70	40	60
90	0	100
95	0	100
95.1	100	0
110	100	0

Diluent: Acetonitrile and *Buffer* (10:90)

System suitability solution: 2 mg/mL of USP Paroxetine Hydrochloride RS, 0.01 mg/mL of USP Paroxetine Related Compound B RS, 0.01 mg/mL of USP Paroxetine Related Compound F RS, and 0.004 mg/mL of USP Paroxetine Related Compound G RS in *Diluent*

Standard solution: 0.004 mg/mL of USP Paroxetine Hydrochloride RS, 0.01 mg/mL of USP Paroxetine Related Compound B RS, 0.01 mg/mL of USP Paroxetine Related Compound F RS, and 0.004 mg/mL of USP Paroxetine Related Compound G RS in *Diluent*

Sample solution: 0.5 mg/mL of Paroxetine Hydrochloride in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 15-cm; packing L1

Flow rate: 1.0 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

[NOTE—Identify the peaks using the relative retention times given in Table 5.]

Suitability requirements

Relative standard deviation: NMT 10.0% for each of paroxetine related compound B, paroxetine related compound F, paroxetine hydrochloride, and paroxetine related compound G

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of paroxetine related compound B, paroxetine related compound F, and paroxetine related compound G in the portion of Paroxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of the corresponding impurity from the *Sample solution*

r_S = peak response of the corresponding impurity from the *Standard solution*

C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

C_U = concentration of Paroxetine Hydrochloride, on the anhydrous basis, in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Paroxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of any individual unspecified impurity from the *Sample solution*

r_S = peak response of paroxetine from the *Standard solution*

C_S = concentration of USP Paroxetine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Paroxetine Hydrochloride, on the anhydrous basis, in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 5.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Paroxetine related compound B	0.91	0.5
Paroxetine related compound F	0.96	0.2
Paroxetine	1.0	—
Paroxetine related compound G	1.34	0.2
Any unspecified impurity	—	0.1
Total impurities	—	1.0

SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 1.5% for the anhydrous form; 2.2%–2.8% for the hemihydrate form

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve the anhydrous form in tight containers. Preserve the hemihydrate form in well-closed containers. Store at controlled room temperature.

- **LABELING:** Label the article to indicate whether it is the anhydrous form or the hemihydrate form, and label it to indicate with which impurity tests the article complies.

• USP REFERENCE STANDARDS (11)

USP Paroxetine Hydrochloride RS

USP Paroxetine Related Compound B RS

trans-4-Phenyl-3-[(3,4-methylenedioxy)phenoxy]methylpiperidine hydrochloride.

$C_{19}H_{21}NO_3 \cdot HCl$ 347.84

USP Paroxetine Related Compound C RS

(+)-*trans*-Paroxetine hydrochloride.

$C_{19}H_{20}FNO_3 \cdot HCl$ 365.83

USP Paroxetine Related Compound E Mixture RS

Paroxetine hydrochloride spiked with 1-methyl-4-(*p*-fluorophenyl)-1,2,3,6-tetrahydropyridine.

USP Paroxetine Related Compound F RS

trans(-)-1-Methyl-3-[1,3-benzodioxol-5-yloxy)methyl]-4-(fluorophenyl)piperidine.

$C_{20}H_{22}FNO_3$ 343.39

USP Paroxetine Related Compound G RS

(±)-*trans*-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4'-fluorophenyl)-4'-phenylpiperidine hydrochloride.

$C_{25}H_{24}FNO_3 \cdot HCl$ 405.46

USP Paroxetine System Suitability Mixture A RS
Mixture of approximately 1% paroxetine related compound A [piperidine, 3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-methoxyphenyl)-, hydrochloride (3*S-trans*)]; and 1% of paroxetine related compound B [piperidine, 3-[(1,3-benzodioxol-5-yloxy)methyl]-4-phenyl-, hydrochloride (3*S-trans*)] in a matrix of paroxetine hydrochloride.

Paroxetine Tablets

DEFINITION

Paroxetine Tablets contain an amount of Paroxetine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$).

IDENTIFICATION

A. INFRARED ABSORPTION (197K)

Sample: Transfer a quantity of finely powdered Tablets, equivalent to 90 mg of paroxetine, to a suitable flask. Add 100 mL of 0.1 N hydrochloric acid, and stir for 1 h. Transfer the mixture to a separatory funnel, and add 1.5 mL of ammonium hydroxide to make the solution alkaline. Add 100 mL of ethyl ether to the funnel, and shake for 2 min. Transfer the organic layer into the necessary number of centrifuge tubes, and centrifuge for 10 min. Recombine the clarified extracts, add 1 drop of water and 0.5 mL of hydrochloric acid, stir, and evaporate to dryness under a stream of nitrogen. Dry the residue in an oven at 90° for 1 h.

Acceptance criteria: Meet the requirements

B. The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Buffer: Water, phosphoric acid, and triethylamine (100:0.6:0.3)

Mobile phase: Acetonitrile and Buffer (30:70)

Standard solution: 0.1 mg/mL of USP Paroxetine Hydrochloride RS in methanol

Sample stock solution: 0.5 mg/mL of paroxetine from NLT 20 Tablets in methanol prepared as follows. Transfer an amount of finely powdered Tablets equivalent to NLT 100 mg of paroxetine to a suitable volumetric flask. Dissolve in methanol. Dilute with methanol to volume. Centrifuge a portion of the solution for 6 min. Use the supernatant.

Sample solution: Nominally 0.1 mg/mL of paroxetine in methanol, from the *Sample stock solution*

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 295 nm

Column: 4.6-mm × 3.3-cm; 3-μm packing L7

Flow rate: 2 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 750 theoretical plates

Tailing factor: NMT 4

Relative standard deviation: NMT 2.0% for paroxetine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = nominal concentration of paroxetine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of paroxetine, 329.37

M_{r2} = molecular weight of paroxetine hydrochloride, 365.83

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: Simulated gastric fluid without enzyme; 900 mL

Apparatus 2: 60 rpm

Time: 60 min

Buffer and Mobile phase: Proceed as directed in the Assay.

Standard stock solution: 0.63 mg/mL of USP Paroxetine Hydrochloride RS in *Medium* prepared as follows. Transfer a suitable quantity of USP Paroxetine Hydrochloride RS to a suitable volumetric flask. Add 5% of the flask volume of methanol, and dissolve. Dilute with *Medium* to volume.

Sample solution: Pass the solution under test through a suitable membrane filter of 0.45-μm pore size.

Standard solution: Quantitatively dilute the *Standard stock solution* with *Medium* to a concentration near that of the *Sample solution*.

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 295 nm

Column: 4.6-mm × 3.3-cm; 3-μm packing L7

Flow rate: 2 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 750 theoretical plates

Tailing factor: NMT 4

Relative standard deviation: NMT 2.0% for paroxetine

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved based on the peak responses obtained from the *Sample solution* and the *Standard solution*.

Tolerances: NLT 80% (Q) of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) is dissolved.

UNIFORMITY OF DOSAGE UNITS (905)

Buffer, Mobile phase, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Sample solution: Nominally 0.1 mg/mL of paroxetine prepared as follows. Place 1 Tablet in a suitable volumetric flask, and add a volume of a hydrochloric acid solution (7 in 1000), equivalent to about 25% of the flask volume. Allow the Tablet to disintegrate. Dilute with methanol to volume. Centrifuge a portion of the solution.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

M_{r1} = molecular weight of paroxetine, 329.37

M_{12} = molecular weight of paroxetine hydrochloride, 365.83

V = volume of the *Sample solution* (mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Paroxetine Hydrochloride RS

Paroxetine Extended-Release Tablets

DEFINITION

Paroxetine Extended-Release Tablets contain paroxetine hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer: 3.9 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 4.5.

Mobile phase: Acetonitrile, *Buffer*, and triethylamine (40:60:1). Adjust with glacial acetic acid to a pH of 5.5.

Standard solution: 0.5 mg/mL of USP Paroxetine Hydrochloride RS in methanol

System suitability solution: 0.5 mg/mL of USP Paroxetine Related Compound B RS in *Standard solution*

Sample solution: Nominally 0.5 mg/mL of paroxetine from NLT 10 Tablets prepared as follows. Transfer the required number of Tablets to a suitable volumetric flask. Add 80% of the flask volume of methanol. Sonicate for 30 min followed by stirring for 30 min. Dilute with methanol to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 295 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L13

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for paroxetine related compound B and paroxetine are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between paroxetine related compound B and paroxetine, *System suitability solution*

Tailing factor: NMT 2.0 for paroxetine, *System suitability solution*

Relative standard deviation: NMT 2.0% for paroxetine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{11}/M_{12}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Paroxetine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of paroxetine in the *Sample solution* (mg/mL)

M_{11} = molecular weight of paroxetine, 329.37

M_{12} = molecular weight of paroxetine hydrochloride, 365.83

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Acid stage medium: 0.1 N hydrochloric acid; 750 mL
Buffer stage medium: 0.05 M tris buffer prepared as follows. Dissolve 6.06 g of tris(hydroxymethyl)amino-methane in 1 L of water. Add 1.8 mL of hydrochloric acid to the resulting solution. Adjust with hydrochloric acid to a pH of 7.5; 1000 mL deaerated.

Apparatus 1: 100 rpm

Times: 2 h in *Acid stage*; 2, 4, and 12 h in *Buffer stage*
Buffer and Mobile phase: Proceed as directed in the *Assay*.

Acid stage standard stock solution: 0.33 mg/mL of paroxetine prepared as follows. Transfer a suitable amount of USP Paroxetine Hydrochloride RS to a suitable volumetric flask. Dissolve in 5% of the flask volume of methanol. Dilute with *Acid stage medium* to volume.

Acid stage standard solution: Dilute the *Acid stage standard stock solution* with *Acid stage medium* to obtain a final concentration of ($L/7500$) mg/mL, where L is the label claim in mg.

Buffer stage standard stock solution: 0.25 mg/mL of paroxetine prepared as follows. Transfer a suitable amount of USP Paroxetine Hydrochloride RS to a suitable volumetric flask. Dissolve in 5% of the flask volume of methanol. Dilute with *Buffer stage medium* to volume.

Buffer stage standard solution: Dilute the *Buffer stage standard stock solution* with *Buffer stage medium* to obtain a final concentration of ($L/1000$) mg/mL, where L is the label claim in mg.

Acid stage sample solution: Run the *Acid stage* for 2 h. Withdraw 10 mL of the solution under test and centrifuge. Use the centrifugate for analysis.

Buffer stage sample solution: Remove the *Acid stage medium* from the vessel and replace it with the *Buffer stage medium*. At the times specified, remove 10 mL of the solution under test and centrifuge. Use the centrifugate for analysis.

Chromatographic system: Proceed as directed in the *Assay*. For *Injection volume*, use 100 μ L for the *Acid stage* analysis and 10 μ L for the *Buffer stage* analysis.

System suitability

Samples: *Acid stage standard solution* and *Buffer stage standard solution*

Suitability requirements

Tailing factor: NMT 2.0, *Acid stage standard solution* and *Buffer stage standard solution*

Relative standard deviation: NMT 3.0%, *Acid stage standard solution* and *Buffer stage standard solution*

Analysis

Samples: *Acid stage standard solution*, *Buffer stage standard solution*, *Acid stage sample solution*, and *Buffer stage sample solution*

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved in the *Acid stage*:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{11}/M_{12}) \times V \times (1/L) \times 100$$

r_U = peak response from the *Acid stage sample solution*

r_S = peak response from the *Acid stage standard solution*

- C_5 = concentration of USP Paroxetine Hydrochloride RS in the *Acid stage standard solution* (mg/mL)
 M_{r1} = molecular weight of paroxetine, 329.37
 M_{r2} = molecular weight of paroxetine hydrochloride, 365.83
 V = volume of the *Acid stage medium* (750 mL)
 L = label claim (mg/Tablet)

Calculate the concentration (C_i) of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at each time point in the *Buffer stage*:

$$\text{Result} = (r_i/r_s) \times C_5 \times (M_{r1}/M_{r2})$$

- r_i = peak response from the *Buffer stage sample solution* at each time point i
 r_s = peak response from the *Buffer stage standard solution*
 C_5 = concentration of USP Paroxetine Hydrochloride RS in the *Buffer stage standard solution* (mg/mL)
 M_{r1} = molecular weight of paroxetine, 329.37
 M_{r2} = molecular weight of paroxetine hydrochloride, 365.83

Calculate the percentage of the labeled amount (Q_i) of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at each time point (i) in the *Buffer stage medium*:

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_s)] + (C_1 \times V_s)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_s)]] + [(C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

- C_i = concentration of paroxetine in the *Buffer stage medium* in the portion of sample withdrawn at time point i (mg/mL)
 V = volume of the *Buffer stage medium*, 1000 mL
 L = label claim (mg/Tablet)
 V_s = volume of the *Sample solution* withdrawn from the *Buffer stage medium* (mL)

Tolerances

Acid stage: NMT 10% of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) is dissolved in 2 h.

Buffer stage: See Table 1.

Table 1

Time point (h)	Time (h)	Amount Dissolved (Tablets labeled to contain 12.5 mg of paroxetine)	Amount Dissolved (Tablets labeled to contain 25 mg of paroxetine)	Amount Dissolved (Tablets labeled to contain 37.5 mg of paroxetine)
1	2	15%–35%	10%–30%	20%–45%
2	4	40%–70%	40%–70%	60%–85%
3	12	NLT 80%	NLT 80%	NLT 80%

The percentages of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

Test 2

If the product complies with this procedure, the labeling indicates that it meets USP *Dissolution Test 2*.

Acid stage medium: 0.1 N hydrochloric acid; 750 mL

Buffer stage medium: 0.05 M tris buffer prepared as follows. Dissolve 6.06 g of tris(hydroxymethyl)amino-methane in 900 mL of water. Add 40 mL of 1.0 M hydrochloric acid to the resulting solution. Adjust with either 1.0 M hydrochloric acid or 1.0 M sodium hy-

droxide to a pH of 7.5. Dilute with water to 1 L; 1000 mL deaerated.

Apparatus 2: 150 rpm with suitable sinkers

Times: 2 h in *Acid stage*; 1, 2, 4, and 6 h in *Buffer stage*

Acid stage standard stock solution: 0.04 mg/mL of USP Paroxetine Hydrochloride RS prepared as follows. Transfer a suitable amount of USP Paroxetine Hydrochloride RS to a suitable volumetric flask. Dissolve in 2 mL of methanol. Dilute with *Acid stage medium* to volume.

Acid stage standard solution: Dilute the *Acid stage standard stock solution* with *Acid stage medium* to obtain a final concentration of $(L/7500)$ mg/mL of paroxetine, where L is the label claim in mg.

Buffer stage standard solution: $(L/1000)$ mg/mL of paroxetine, where L is the label claim in mg prepared as follows. Transfer a suitable amount of USP Paroxetine Hydrochloride RS to a suitable volumetric flask. Dissolve in 2 mL of methanol. Dilute with *Buffer stage medium* to volume.

Acid stage sample solution: Run the *Acid stage* for 2 h. Withdraw 10 mL of the solution under test, and filter. Use the filtrate for analysis.

Buffer stage sample solution: Remove the Tablet and sinker from the acid stage vessel and pat them dry. Introduce the Tablet and sinker into the dissolution vessel with 1000 mL of the *Buffer stage medium*. At the times specified, remove 10 mL of the solution under test and filter. Use the filtrate for analysis.

Acid stage analysis

Buffer: Add 2.9 mL of phosphoric acid to 800 mL of water. Adjust with 1 M sodium hydroxide to a pH of 6.0. Dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (40:60)

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 2 mL/min

Injection volume: 50 μ L for 12.5-mg Tablet; 20 μ L for 25- and 37.5-mg Tablets

Run time: 2 times the retention time of paroxetine

System suitability

Sample: *Acid stage standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Acid stage standard solution* and *Acid stage sample solution*

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved in the *Acid stage*:

$$\text{Result} = (r_u/r_s) \times C_5 \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

r_u = peak response from the *Acid stage sample solution*

r_s = peak response from the *Acid stage standard solution*

C_5 = concentration of USP Paroxetine Hydrochloride RS in the *Acid stage standard solution* (mg/mL)

M_{r1} = molecular weight of paroxetine, 329.37

M_{r2} = molecular weight of paroxetine hydrochloride, 365.83

V = volume of the *Acid stage medium* (750 mL)

L = label claim (mg/Tablet)

Buffer stage analysis

Instrumental conditions

Mode: UV

Analytical wavelength: 294 nm with 340 nm for background correction

Blank: Buffer stage medium

Analysis

Samples: Buffer stage standard solution and Buffer stage sample solution

Calculate the concentration (C_i) of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at each time point (i) in the Buffer stage:

$$\text{Result} = (A_i/A_s) \times C_s \times (M_{r1}/M_{r2})$$

A_i = absorbance of the Buffer stage sample solution at time point i

A_s = absorbance of the Buffer stage standard solution

C_s = concentration of USP Paroxetine Hydrochloride RS in the Buffer stage standard solution (mg/mL)

M_{r1} = molecular weight of paroxetine, 329.37

M_{r2} = molecular weight of paroxetine hydrochloride, 365.83

Calculate the percentage of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at each time point (i) in the Buffer stage medium:

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times (V - V_3)) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = [(C_3 \times [V - (2 \times V_3)]) + [(C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

$$\text{Result}_4 = [(C_4 \times [V - (3 \times V_3)]) + [(C_3 + C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

C_i = concentration of paroxetine in the Buffer stage medium in the portion of sample withdrawn at time point i (mg/mL)

V = volume of the Buffer stage medium, 1000 mL

L = label claim (mg/Tablet)

V_3 = volume of the Sample solution withdrawn from the Buffer stage medium (mL)

Tolerances

Acid stage: NMT 10% of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) is dissolved in 2 h.

Buffer stage: See Table 2.

Table 2

Time Point (i)	Time (h)	Amount Dissolved
1	1	NMT 20%
2	2	20%–45%
3	4	60%–90%
4	6	NLT 85%

The percentages of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$) dissolved at the times specified conform to Dissolution (711), Acceptance Table 2.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Solution A: Tetrahydrofuran, water, and trifluoroacetic acid (20:180:1)

Solution B: Acetonitrile, tetrahydrofuran, and trifluoroacetic acid (180:20:1)

Mobile phase: See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	80	20
30	80	20
50	20	80
60	20	80
70	80	20
80	80	20

System suitability solution: 1 mg/mL of USP Paroxetine Hydrochloride RS, 0.1 mg/mL of USP Paroxetine System Suitability Mixture A RS, and 1 mg/mL of USP Paroxetine Related Compound F RS in methanol.

[NOTE—Sonication may be used to aid dissolution of the individual components.]

Standard solution: 0.01 mg/mL of USP Paroxetine Hydrochloride RS in methanol

Sample solution: Nominally 1 mg/mL of paroxetine from NLT 10 Tablets prepared as follows. Transfer a suitable number of Tablets to a suitable volumetric flask. Add 50% of the flask volume of methanol. Sonicate for 30 min followed by stirring for 30 min. Dilute with methanol to volume. Mix and centrifuge. Use the clear centrifugate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L7

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Samples: System suitability solution and Standard solution

[NOTE—See Table 4 for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between paroxetine related compound A and paroxetine related compound B; NLT 1.5 between paroxetine related compound F and paroxetine, System suitability solution

Tailing factor: NMT 2.0 for paroxetine, Standard solution

Relative standard deviation: NMT 5.0% for paroxetine, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Paroxetine Hydrochloride RS in the Standard solution (mg/mL)

C_U = nominal concentration of paroxetine in the Sample solution (mg/mL)

M_{r1} = molecular weight of paroxetine 329.37

M_{r2} = molecular weight of paroxetine hydrochloride, 365.83

Acceptance criteria: See Table 4.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Paroxetine related compound A ^a	0.67	—
Paroxetine related compound B ^a	0.75	—
Paroxetine related compound F ^a	0.90	—
Paroxetine	1.0	—
Ethoxyparoxetine ^b	1.2	0.2
Any unspecified degradation product	—	0.2
Total impurities	—	0.5

^a Process impurities, included for identification only. Process impurities are controlled in the drug substance and are not to be reported or included in the total impurities of the drug product.

^b (3*SR*,4*RS*)-3-(1,3-Benzodioxol-5-yloxy)methyl)-4-(4-ethoxyphenyl)piperidine.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature.
- **LABELING** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

Change to read:

• USP REFERENCE STANDARDS (11)

USP Paroxetine Hydrochloride RS

USP Paroxetine Related Compound B RS

trans-4-Phenyl-3-[(3,4-methylenedioxy)phenoxy]methylpiperidine hydrochloride; also known as Piperidine, 3-[(1,3-benzodioxol-5-yloxy)methyl]-4-phenyl-, hydrochloride (3*S-trans*).
 $C_{19}H_{21}NO_3 \cdot HCl$ 347.84 (ERR 1-Jun-2016)

USP Paroxetine Related Compound F RS

trans(-)-1-Methyl-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine.
 $C_{20}H_{22}FNO_3$ 343.39

USP Paroxetine System Suitability Mixture A RS

Mixture of approximately 1% paroxetine related compound A [piperidine, 3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-methoxyphenyl)-, hydrochloride (3*S-trans*)], and 1% paroxetine related compound B [piperidine, 3-[(1,3-benzodioxol-5-yloxy)methyl]-4-phenyl-, hydrochloride (3*S-trans*)] in a matrix of paroxetine hydrochloride.

yields NLT 74.0% of galacturonic acid ($C_6H_{10}O_7$), calculated on the dried basis.

[NOTE—Commercial pectin for the production of jellied food products is standardized to the convenient “150 jelly grade” by addition of dextrose or other sugars, and sometimes contains sodium citrate or other buffer salts. This monograph refers to the pectin to which no such additions have been made.]

IDENTIFICATION

• PROCEDURE

Sample stock solution: Transfer a quantity of Pectin, equivalent to 0.05 g on the dried basis, to a suitable container, and moisten with 250 μ L of 2-propanol. Add 50 mL of water to the container, and mix the solution using a magnetic stirrer. Use 0.5 N sodium hydroxide to adjust the pH of the solution to 12, stop the stirrer, and allow the solution to stand undisturbed at room temperature for 15 min. Adjust with 0.5 N hydrochloric acid to a pH of 7.0, and dilute with water to 100 mL.

Tris buffer solution: Transfer 6.055 g of tris(hydroxymethyl)aminomethane and 0.147 g of calcium chloride ($CaCl_2 \cdot 2H_2O$) to a 1000-mL volumetric flask containing 950 mL of water. Adjust with 1 N hydrochloric acid to a pH of 7.0, and dilute with water to volume.

Enzyme solution: Mix pectate lyase¹ with *Tris buffer solution* to make a solution (1 in 100).

Sample blank: Mix 0.5 mL of *Tris buffer solution*, 1.0 mL of *Sample stock solution*, and 1.0 mL of water in a quartz cuvette.

Enzyme blank: Mix 0.5 mL of *Tris buffer solution*, 1.5 mL of water, and 0.5 mL of *Enzyme solution* in a quartz cuvette.

Sample solution: Mix 0.5 mL of *Tris buffer solution*, 1.0 mL of *Sample stock solution*, 0.5 mL of water, and 0.5 mL of *Enzyme solution* in a quartz cuvette.

Analysis

Samples: *Sample blank*, *Enzyme blank*, and *Sample solution*

Perform the test with the *Samples* using a suitable UV/visible spectrophotometer (see *Ultraviolet-Visible Spectroscopy* (857)) and using water as a blank. Measure the absorbance at 235 nm immediately after mixing the solutions well, and record the value at time 0 for the *Enzyme blank*, A_{0-EB} ; for the *Sample blank*, A_{0-TB} ; and for the *Sample solution*, A_{0-TS} . After incubation at room temperature for 10 min, determine the absorbance again at 235 nm for the *Enzyme blank*, A_{10-EB} ; for the *Sample blank*, A_{10-TB} ; and for the *Sample solution*, A_{10-TS} . Calculate the corrected absorbance A_0 at time 0 and the corrected absorbance A_{10} at 10 min:

$$A_0 = A_{0-TS} - (A_{0-EB} + A_{0-TB})$$

$$A_{10} = A_{10-TS} - (A_{10-EB} + A_{10-TB})$$

Calculate the quantity of unsaturated product produced:

$$\text{Result} = (A_{10} - A_0) / (\epsilon_{235} \times L)$$

ϵ_{235} = molar extinction coefficient of the reaction product ($4600 \text{ M}^{-1} \cdot \text{cm}^{-1}$)

L = path length of the reaction cuvette, 1 cm

Acceptance criteria: The amount of unsaturated product is NLT $0.5 \times 10^{-5} \text{ M}$.

ASSAY

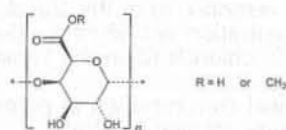
• DEGREE OF ESTERIFICATION

Sample: 5.0 g

Analysis: Transfer the *Sample* to a suitable beaker, and stir for 10 min with a mixture of 5 mL of hydrochloric acid and 100 mL of 60% alcohol. Transfer to a sintered-

¹ A suitable pure enzyme is available from Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland (www.megazyme.com).

Pectin



Pectin [9000-69-5].

DEFINITION

Pectin is a purified carbohydrate polymer consisting mainly of a linear backbone of partially methoxylated alpha (1-4) linked D-galacturonic acid. It is obtained from the dilute acid extract of the rind of citrus fruits or from apple pomace. No organic solvents other than methanol, ethanol, and isopropanol are used during its production. Pectin

glass filter (30- to 60-mL crucible or Büchner type, coarse), and wash with six 15-mL portions of the hydrochloric acid–60% alcohol mixture, followed by 60% alcohol until the filtrate is free from chlorides. Finally wash with 20 mL of alcohol, dry for 1 h at 105°, cool, and weigh. Transfer exactly one-tenth of the total net weight of the dried *Sample* (representing 500 mg of the original unwashed *Sample*) to a 250-mL conical flask, and moisten with 2 mL of alcohol. Add 100 mL of carbon dioxide-free water, insert the stopper, and swirl occasionally until the Pectin is completely dissolved. Add 5 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Perform a blank determination, and make any necessary correction. Record the results as the initial titer, V_i (mL). Add 20.0 mL of 0.5 N sodium hydroxide VS, insert the stopper, shake vigorously, and allow to stand for 15 min. Add 20.0 mL of 0.5 N hydrochloric acid VS, and shake until the pink color disappears. Add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS to a faint pink color that persists after vigorous shaking. Perform a blank determination, and make any necessary correction. Record this value as the saponification titer, V_s (mL). Calculate the degree of esterification:

$$\text{Result} = [V_s / (V_i + V_s)] \times 100$$

V_s = saponification titer (mL)

V_i = initial titer (mL)

Acceptance criteria: The value for *Degree of Esterification* is within the range stated on the label.

- **GALACTURONIC ACID:** Each mL of 0.1 N sodium hydroxide used in the total titration (the initial titer added to the saponification titer) in the *Assay for Degree of Esterification* is equivalent to 19.41 mg of $C_6H_{10}O_7$. Calculate the percentage of galacturonic acid in the portion of Pectin taken:

$$\text{Result} = 19.41 \times [(V_i + V_s) / W] \times 100$$

V_i = initial titer (mL)

V_s = saponification titer (mL)

W = weight of the original unwashed and dried Pectin taken to prepare the solution for titration (mg)

Acceptance criteria: NLT 74.0%

- **METHOXY GROUPS:** Each mL of 0.1 N sodium hydroxide used in the saponification titer in the *Assay for Degree of Esterification* is equivalent to 3.10 mg of $-OCH_3$. Calculate the percentage of methoxy groups in the portion of Pectin taken:

$$\text{Result} = 3.10 \times (V_s / W) \times 100$$

V_s = saponification titer (mL)

W = weight of the original unwashed and dried Pectin taken to prepare the solution for titration (mg)

Acceptance criteria: The percentage of methoxy groups is within the range stated on the label.

IMPURITIES

- **ARSENIC, Method II (211):** NMT 3 ppm

- **LEAD**

Standard stock solution: 1000 µg/mL of lead. [NOTE—Use a commercially available certified solution.]

Standard solution: 2 µg/mL of lead, prepared immediately before use by pipetting 0.10 mL of *Standard stock solution* into a 50-mL volumetric flask containing 30 mL of water, 4 mL of 20% hydrochloric acid, and 4 mL of 0.1 M EDTA. Dilute with water to volume, and mix.

Reference solution: 0.4 µg/mL of lead, prepared by pipetting 5.0 mL of the *Standard solution* into a 25-mL volumetric flask containing 10 mL of water, 2 mL of 20% hydrochloric acid, and 2 mL of 0.1 M EDTA. Dilute with water to volume, and mix.

Sample solution: Transfer 2.0 g of Pectin to a clean, 100-mL glass beaker, add 25 mL of 70% nitric acid, cover with a watch glass, and heat at low to moderate heat on a hot plate in a fume hood for 2 h. Remove the watch glass, and continue to heat until the sample is dry with no visible fumes. Add 0.5 mL of 70% nitric acid, and heat to dryness. Cool to room temperature, and add 2 mL of 20% hydrochloric acid and 2 mL of 0.1 M EDTA. Quantitatively transfer the solution to a 25-mL volumetric flask, dilute with water to volume, and mix.

Blank solution: Add 30 mL of water, 4 mL of 20% hydrochloric acid, and 4 mL of 0.1 M EDTA into a 50-mL volumetric flask. Dilute with water to volume, and mix.

Analysis: Lead is determined using an inductively coupled plasma–atomic emission spectrometer (ICP–AES) (see *Plasma Spectrochemistry* (730)) by measuring the emission at 220.35 nm with the settings optimized as directed by the manufacturer. Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check. Calibrate the instrument with the *Blank solution* and the *Standard solution*. Then analyze the *Reference solution* and the *Sample solution*.

Acceptance criteria: The concentration in the *Sample solution* is NMT that in the *Reference solution*, corresponding to NMT 5 ppm of lead.

- **SULFUR DIOXIDE, Method V (525)**

Sample: 100 g

Analysis: Suspend the *Sample* in 500 mL of methanol, then transfer this mixture to the flask (C). Prepare a mixture of 20 mL of hydrochloric acid and 10 mL of water, and transfer it to the separatory funnel (B). Add 10 mL of hydrogen peroxide solution to the vessel (G). Perform the refluxing for 2 h before removing the vessel (G).

Acceptance criteria: NMT 50 ppm

- **SUGARS AND ORGANIC ACIDS**

Sample: 1 g

Analysis: Place the *Sample* in a 500-mL flask, moisten with 3–5 mL of alcohol, rapidly pour in 100 mL of water, shake, and allow to stand until the solution is complete. To this solution add 100 mL of alcohol containing 0.3 mL of hydrochloric acid, mix, and filter rapidly. Measure 25 mL of the filtrate into a tared dish, evaporate the liquid on a steam bath, and dry the residue in a vacuum oven at 50° for 2 h.

Acceptance criteria: The weight of the residue does not exceed 20 mg, corresponding to NMT 16% of sugars and organic acids.

- **METHANOL (METHYL ALCOHOL), ETHANOL (ALCOHOL), AND ISOPROPANOL (2-PROPANOL)**

[NOTE—Residual alcohols are volatile. They should be stored in a cool and dry place. When preparing the *Standard stock solution*, *Standard solution*, and *Internal standard stock solution* for residual alcohols, mix thoroughly and keep the solutions at 20° when diluting with water to volume.]

Internal standard stock solution: 5000 µg/mL of USP 2-Butanol RS. [NOTE—This solution can be stored at 5°–8° for 3 months.]

Standard stock solution: Use USP Methyl Alcohol RS, USP 2-Propanol RS, and alcohol to prepare a solution having known concentrations of 5000 µg/mL each for methyl alcohol, 2-propanol, and alcohol.

Standard solution: To a 250-mL volumetric flask add 2.5 mL of the *Standard stock solution* and 2.5 mL of the *Internal standard stock solution*. Dilute with water to volume, and mix. This solution contains 50 µg/mL each of methyl alcohol, 2-propanol, and alcohol. [NOTE—This solution can be stored at 5°–8° for 3 months.] Transfer 1.0 g of this solution to a 10-mL headspace vial.

Sample solution: Transfer 1.0 g of Pectin and 5 g of sucrose to a stoppered 100-mL Erlenmeyer flask con-

taining 90 mL of water, add 1.0 mL of the *Internal standard stock solution*, and dilute with water to 100 mL. Mix the solution using a magnetic stirrer. Continue stirring until all of the Pectin has been completely dissolved; typically it takes about 1–2 h. This solution contains 50 µg/mL of USP 2-Butanol RS. Transfer 1.0 g of this solution to a 10-mL headspace vial.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: Headspace GC

Detector: Flame ionization

Column: 0.32-mm × 30-m capillary column; 1.8-µm layer of phase G43. [NOTE—An alternative column such as a 0.32-mm × 25-m capillary column bonded with a 5-µm layer of phase S3 can be used as long as the system suitability requirements are met.]

Temperatures

Detector: 280°

Column: 70°

Injection port: 200°

Carrier gas: Nitrogen

Flow rate: 1.5 mL/min

Make up gas: Nitrogen

Split flow rate: 30 mL/min

Injection volume: 1 mL (the gaseous headspace)

Injection type: Split ratio 20:1

Balanced pressure automatic headspace sampler

Equilibration time: 10 min

Equilibration temperature: 70°

Agitation speed: 500 rpm

Agitation on time: 5 s

Agitation off time: 90 s

Syringe temperature: 80°

Syringe size: 2.5 mL

Fill speed: 100 µL/s

Pull-up delay: 2.0 s

GC run time: 10.5 min

[NOTE—These GC conditions should be optimized according to the instruments used.]

System suitability

Sample: *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Table 1

Component	Relative Retention Time
Methanol	0.5
Ethanol	0.6
2-Propanol	0.7
2-Butanol	1.0

Suitability requirements

Resolution: NLT 1.5, between each pair of analytes

Relative standard deviation: NMT 10%, determined from each analyte

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methanol, ethanol, and 2-propanol in the portion of Pectin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/W) \times F \times V \times 100$$

R_U = internal standard ratio (peak response of the respective alcohol/peak response of the internal standard) from the *Sample solution*

R_S = internal standard ratio (peak response of the respective alcohol/peak response of the internal standard) from the *Standard solution*

C_S = concentration of the respective residual alcohol (methanol, ethanol, or 2-propanol) in the *Standard solution* (µg/mL)

W = weight of Pectin taken to prepare the *Sample solution* (g)

F = conversion factor (10^{-6} g/µg)

V = volume of the *Sample solution*, 100 mL

Acceptance criteria: NMT 1% for total methanol, ethanol, and isopropanol

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count is NMT 10^3 cfu/g, and the total combined molds and yeasts count is NMT 10^2 cfu/g.

• **LOSS ON DRYING** (731)

Analysis: Dry a sample at 105° for 3 h.

Acceptance criteria: NMT 10.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store in a cool and dry place.

• **LABELING:** Label it to indicate whether it is of apple or of citrus origin. Label it to indicate the range of the degree of esterification and the range of the percentage of methoxy groups. The labeling also indicates the presence of sulfur dioxide if the residual sulfur dioxide concentration is greater than 10 ppm.

• **USP REFERENCE STANDARDS** (11)

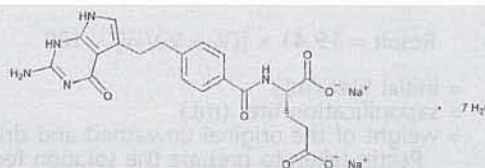
USP 2-Butanol RS

USP Methyl Alcohol RS

USP 2-Propanol RS

Add the following:

▲Pemetrexed Disodium



$C_{20}H_{19}N_5Na_2O_6 \cdot 7H_2O$ 597.49

L-Glutamic acid, N-[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-, disodium salt, heptahydrate;

Disodium N-[p-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamate heptahydrate [357166-29-1].

$C_{20}H_{19}N_5Na_2O_6$ 471.37

Anhydrous [150399-23-8].

$C_{20}H_{21}N_5O_6$ 427.42

Pemetrexed (free acid) [137281-23-3].

DEFINITION

Pemetrexed Disodium contains NLT 97.5% and NMT 102.0% of pemetrexed disodium ($C_{20}H_{19}N_5Na_2O_6$), calculated on the anhydrous and solvent-free basis.

[CAUTION]—Handle pemetrexed disodium with great care as it alters genetic material and may be irritating to the eyes and skin.]

IDENTIFICATION

• **A. INFRARED ABSORPTION** (197): [NOTE—Methods described in (197K) or (197A) may be used.]

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Enantiomeric Purity* test.

• **C. IDENTIFICATION TESTS—GENERAL** (191), *Sodium*

ASSAY

• **PROCEDURE**

Buffer: 0.17% (v/v) glacial acetic acid in water. Adjust with a 50% sodium hydroxide solution to a pH of 5.3 ± 0.1.

Mobile phase: Acetonitrile and *Buffer* (11:89)

Standard solution: 0.15 mg/mL of USP Pemetrexed Disodium RS in water

Sample solution: 0.15 mg/mL of Pemetrexed Disodium in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 285 nm

Column: 4.6-mm × 7.5-cm; 3.5-μm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pemetrexed disodium ($C_{20}H_{19}N_5Na_2O_6$) in the portion of Pemetrexed Disodium taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Pemetrexed Disodium RS in the *Standard solution* (mg/mL)

C_u = concentration of Pemetrexed Disodium in the *Sample solution* (mg/mL)

Acceptance criteria: 97.5%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer: 1.45 g/L of ammonium formate in water. Adjust with formic acid to a pH of 3.5 ± 0.1.

Solution A: Acetonitrile and *Buffer* (5:95)

Solution B: Acetonitrile and *Buffer* (30:70)

Mobile phase: See *Table 1*. [NOTE—After each injection, re-equilibrate the chromatographic system at the initial condition for a minimum of 13 min.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
45	0	100
47	100	0

System suitability stock solution: Prepare 3 mg/mL of USP Pemetrexed Disodium RS in 0.1 N sodium hydroxide. Heat this solution at 70° for 40 min.

[NOTE—The preparation degrades pemetrexed and generates the pemetrexed *R*-dimer and pemetrexed *S*-dimer as follows:

Pemetrexed *R*-dimer: (2*S*,2'*S*)-2,2'-[(*R*)-2,2'-Diamino-4,4',6-trioxo-1,4,4',6,7,7'-hexahydro-1'*H*,5*H*-5,6'-bipyrolo[2,3-*d*]pyrimidine-5,5'-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino))diglutamic acid.

Pemetrexed *S*-dimer: (2*S*,2'*S*)-2,2'-[(*S*)-2,2'-Diamino-4,4',6-trioxo-1,4,4',6,7,7'-hexahydro-1'*H*,5*H*-5,6'-bipyrolo[2,3-*d*]pyrimidine-5,5'-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino))diglutamic acid.]

System suitability solution: Transfer 1 mL of the *System suitability stock solution* to a 10-mL volumetric flask and dilute with water to volume.

Sensitivity solution: 0.1 μg/mL of USP Pemetrexed Disodium RS in water

Sample solution: 0.2 mg/mL of Pemetrexed Disodium in water. Do not sonicate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L7

Autosampler temperature: 2°–8°

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

[NOTE—The relative retention times for the pemetrexed *R*-dimer and pemetrexed *S*-dimer peaks are 0.87 and 0.88, respectively.]

Suitability requirements

Peak-to-valley ratio: The ratio of the height of the pemetrexed *R*-dimer peak to the height of the valley between the pemetrexed *R*-dimer and pemetrexed *S*-dimer is NLT 1.5, *System suitability solution*.

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Pemetrexed Disodium taken:

$$\text{Result} = (r_u/r_t) \times 100$$

r_u = peak area of each impurity from the *Sample solution*

r_t = total peak areas from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
<i>N</i> -Methyl pemetrexed ^a	0.82	0.15
Pemetrexed glutamide ^b	0.90	0.15
Pemetrexed	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.60

^a [4-{2-(2-Amino-1-methyl-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl}benzoyl]-L-glutamic acid.

^b [4-{2-(2-Amino-1-methyl-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl}benzoyl]-L-glutamic acid.

• **ENANTIOMERIC PURITY**

Buffer: Dissolve 8 g of anhydrous beta cyclodextrin in 1 L of water. Add 15 mL of triethylamine to this solution and mix. Add about 6 mL of phosphoric acid and adjust with additional phosphoric acid to a pH of 6.0.

Mobile phase: Acetonitrile and *Buffer* (5:95)

Standard solution: 0.24 mg/mL of USP Pemetrexed Disodium RS in water

Sensitivity solution: 0.12 μg/mL of USP Pemetrexed Disodium RS in water from the *Standard solution*

Sample solution: 0.24 mg/mL of Pemetrexed Disodium in water

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Temperatures

Autosampler: 2°–8°

Column: 40°

Flow rate: 1 mL/min

Injection volume: 50 μL

System suitability**Samples:** *Standard solution* and *Sensitivity solution*

[NOTE—USP Pemetrexed Disodium RS contains a small amount of pemetrexed enantiomer disodium (disodium *N*-(*p*-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo [2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl)-*D*-glutamate). The relative retention times for pemetrexed enantiomer and pemetrexed are about 0.94 and 1.0, respectively.]

Suitability requirements

Peak-to-valley ratio: The ratio of the height of the pemetrexed enantiomer peak to the height of the valley between the pemetrexed enantiomer and pemetrexed is NLT 5.0, *Standard solution*

Signal-to-noise ratio: NLT 10 for the pemetrexed peak, *Sensitivity solution*

Analysis**Sample:** *Sample solution*

Calculate the percentage of pemetrexed enantiomer in the portion of Pemetrexed Disodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of pemetrexed enantiomer from the *Sample solution*

r_T = total peak areas of pemetrexed enantiomer and pemetrexed from the *Sample solution*

Acceptance criteria: NMT 0.3%

SPECIFIC TESTS• **WATER DETERMINATION** (921), *Method I, Method Ia or**Method Ic*: 19.5%–22.1%• **PH** (791)**Sample:** 56 mg/mL in water

Acceptance criteria: 7.5–8.4

• **BACTERIAL ENDOTOXINS TEST** (85): It contains less than 0.17 USP Endotoxin Units/mg of pemetrexed.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.• **USP REFERENCE STANDARDS** (11)

USP Endotoxin RS

USP Pemetrexed Disodium RS

▲USP40

Add the following:**Pemetrexed for Injection****DEFINITION**

Pemetrexed for Injection is a sterile, lyophilized mixture of pemetrexed disodium and suitable added substances. It contains NLT 90.0% and NMT 110.0% of the labeled amount of pemetrexed ($C_{20}H_{21}N_5O_6$).

[CAUTION—Handle pemetrexed disodium with great care as it alters genetic material and may be irritating to the eyes and skin.]

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Buffer: 0.17% (v/v) glacial acetic acid in water. Adjust with a 50% sodium hydroxide solution to a pH of 5.3.

Mobile phase: Acetonitrile and *Buffer* (11:89)

Standard solution: 0.14 mg/mL of USP Pemetrexed Disodium RS in water

Sample solution: Nominally equivalent to 0.1 mg/mL of pemetrexed in water, prepared from a composite of at least three vials of Pemetrexed for Injection

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 285 nm or diode array. [NOTE—Use a diode array detector to perform *Identification B*.]

Column: 4.6-mm × 7.5-cm; 3.5-μm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability**Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 1.5%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pemetrexed ($C_{20}H_{21}N_5O_6$) in the portion of Pemetrexed for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Pemetrexed Disodium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pemetrexed in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of pemetrexed, 427.42

M_{r2} = molecular weight of pemetrexed disodium, 597.49

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **UNIFORMITY OF DOSAGE UNITS** (905), *Weight Variation*: Meets the requirements**IMPURITIES**• **ORGANIC IMPURITIES**

Buffer: 0.17% (v/v) glacial acetic acid in water. Adjust with a 50% sodium hydroxide solution to a pH of 5.5.

Solution A: Acetonitrile and *Buffer* (3:97)

Solution B: Acetonitrile and *Buffer* (12.5: 87.5)

Mobile phase: See *Table 1*. [NOTE—After each injection, re-equilibrate the chromatographic system at the initial condition for a minimum of 8 min.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	0	100
45	0	100
47	100	0

System suitability stock solution: 2.8 mg/mL of USP Pemetrexed Disodium RS prepared as follows. Transfer USP Pemetrexed Disodium RS to a suitable volumetric flask and add a 3% hydrogen peroxide solution equivalent to 10% of the final volume. Dilute with water to volume. Mix and heat this solution at 75° for 2–5 h and allow it to come to room temperature.

[NOTE—The solution preparation forms ketopemetrexed, pemetrexed R-dimer, and pemetrexed S-dimer.

Pemetrexed R-dimer: (2*S*,2'*S*)-2,2'-[[(*R*)-2,2'-Diamino-4,4',6-trioxo-1,4,4',6,7,7'-hexahydro-1'*H*,5*H*-5,6'-bipyrolo[2,3-*d*]pyrimidine-5,5'-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)]diglutaric acid.

Pemetrexed S-dimer: (2*S*,2'*S*)-2,2'-[[(*S*)-2,2'-Diamino-4,4',6-trioxo-1,4,4',6,7,7'-hexahydro-1'*H*,5*H*-5,6'-bipyrolo[2,3-*d*]pyrimidine-5,5'-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)]diglutaric acid.]

System suitability solution: Transfer 5 mL of the System suitability stock solution to a 50-mL volumetric flask and dilute with water to volume.

Sensitivity solution: 0.14 µg/mL of USP Pemetrexed Disodium RS in water

Sample solution: Nominally equivalent to 0.2 mg/mL of pemetrexed in water, prepared from 1 vial of Pemetrexed for Injection

Chromatographic system
(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Temperatures

Autosampler: 2°–8°

Column: 35°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: System suitability solution and Sensitivity solution

[NOTE—The relative retention times for the pemetrexed R-dimer and pemetrexed S-dimer peaks are 0.67 and 0.71, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the pemetrexed R-dimer and pemetrexed S-dimer peaks, System suitability solution

Signal-to-noise ratio: NLT 10, Sensitivity solution

Analysis

Sample: Sample solution

Calculate the percentage of each impurity in the portion of Pemetrexed for Injection taken:

$$\text{Result} = (r_u/r_t) \times (1/F) \times 100$$

r_u = peak area of each impurity from the Sample solution

r_t = total peak areas from the Sample solution

F = relative response factor for each individual impurity (see Table 2)

Acceptance criteria: See Table 2. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ketopemetrexed ^a	0.31	0.61	0.60
Pemetrexed	1.0	—	—

^a 4-[2-[(*R*)-2-Amino-4,6-dioxo-4,5,6,7-tetrahydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]ethyl]benzoyl]-L-glutamic acid.

Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	1.0	0.24
Total Impurities	—	—	1.30

^a 4-[2-[(*R*)-2-Amino-4,6-dioxo-4,5,6,7-tetrahydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]ethyl]benzoyl]-L-glutamic acid.

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.17 USP Endotoxin Units/mg of pemetrexed

• **STERILITY TESTS (71):** Meets the requirements

• **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections

• **pH (791)**

Sample: A constituted solution prepared as directed in the labeling

Acceptance criteria: 6.6–7.8

• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Sterile solids packaging*. Store at controlled room temperature.

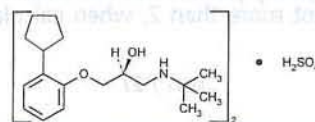
• **USP REFERENCE STANDARDS (11)**

USP Endotoxin RS

USP Pemetrexed Disodium RS

▲USP40

Penbutolol Sulfate



(C₁₈H₂₉NO₂)₂ · H₂SO₄ 680.94

2-Propanol, 1-(2-cyclopentylphenoxy)-3-[(1,1-dimethylethyl)amino]-, (*S*)-, sulfate (2:1) (salt).

(*S*)-1-(*tert*-Butylamino)-3-(*o*-cyclopentylphenoxy)-2-propanol sulfate (2:1) (salt) [38363-32-5].

» Penbutolol Sulfate contains not less than 98.0 percent and not more than 102.0 percent of (C₁₈H₂₉NO₂)₂ · H₂SO₄, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Penbutolol Sulfate RS

Identification—

A: *Infrared Absorption* (197M).

B: A solution (10 mg per mL) responds to the tests for Sulfate (191).

Loss on drying (731)—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.2%.

Specific rotation (781S): between –22° and –26°, determined at 20°.

Test solution: 10 mg per mL, in methanol.

Chromatographic purity—

Organic phase—Prepare a mixture of methanol and acetonitrile (610:390). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Aqueous phase—Dissolve 11 g of sodium 1-heptanesulfonate in 1000 mL of water, add 5.0 mL of triethylamine, adjust with phosphoric acid to a pH of 2.70 ± 0.05 , and filter through a filter having a porosity of 0.5 μm or finer. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Solvent mixture—Prepare a mixture of *Organic phase* and *Aqueous phase* (600:400).

Test solution—Transfer about 50 mg of Penbutolol Sulfate to a 25-mL volumetric flask, dissolve in and dilute with *Solvent mixture* to volume, and mix.

Diluted test solution—Transfer 1.0 mL of the *Test solution* to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 271-nm detector, a preinjection guard column that contains packing L4, and a 4.6-mm \times 25-cm analytical column that contains packing L1 and is maintained at a constant temperature between ambient and 40°, and is programmed to provide variable mixtures of *Organic phase* and *Aqueous phase*. Before each injection, the system is equilibrated with a mobile phase consisting of a mixture of 60% *Organic phase* and 40% *Aqueous phase*. After each injection, this composition of the mobile phase is maintained for 15 minutes, then the proportion of *Organic phase* is increased linearly over the next 20 minutes so that the mobile phase consists of 80% *Organic phase* and 20% *Aqueous phase*. The proportion of *Organic phase* is then decreased to 60% over 1 minute. The flow rate is about 1 mL per minute. Chromatograph the *Diluted test solution*, and record the peak responses as directed for *Procedure*: the resolution, R , between the penbutolol peak and any impurity peak is not less than 1.5, and the tailing factor is not more than 2, when calculated by the formula:

$$W_{0.1} / 2f$$

in which $W_{0.1}$ is the width of the peak at 10% of peak height.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 μL) of the *Solvent mixture*, the *Test solution*, and the *Diluted test solution* into the chromatograph, and measure the peak responses for all the peaks. Calculate the percentage of each individual impurity in the Penbutolol Sulfate taken by the formula:

$$r_i / r_D$$

in which r_i is the peak response for an individual impurity in the chromatogram of the *Test solution*, and r_D is the penbutolol peak response obtained from the *Diluted test solution*: not more than 1.2% of any impurity is found.

Assay—

Organic phase—Prepare a mixture of methanol and acetonitrile (610:390). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Aqueous phase—Dissolve 11 g of sodium 1-heptanesulfonate in 1000 mL of water, add 5.0 mL of triethylamine, adjust with phosphoric acid to a pH of 2.70 ± 0.05 , and filter through a filter having a porosity of 0.5 μm or finer. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Mobile phase—Prepare a mixture of *Organic phase* and *Aqueous phase* (650:350). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard solution—Prepare a solution of 3,4-dimethylbenzophenone in *Mobile phase* containing about 0.01 mg per mL.

Standard preparation—Transfer about 24 mg of USP Penbutolol Sulfate RS, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Internal standard solution* to volume, and mix.

Assay preparation—Transfer about 24 mg of Penbutolol Sulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Internal standard solution* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 271-nm detector and a 4.6-mm \times 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for penbutolol and 1.0 for 3,4-dimethylbenzophenone, and the resolution, R , between the penbutolol peak and the 3,4-dimethylbenzophenone peak is not less than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the responses for the major peaks. Calculate the quantity, in mg, of $(\text{C}_{18}\text{H}_{29}\text{NO}_2)_2 \cdot \text{H}_2\text{SO}_4$ in the portion of Penbutolol Sulfate taken by the formula:

$$100C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Penbutolol Sulfate RS in the *Standard preparation*, and R_U and R_S are the ratios of the penbutolol peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penbutolol Sulfate Tablets

» Penbutolol Sulfate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $(\text{C}_{18}\text{H}_{29}\text{NO}_2)_2 \cdot \text{H}_2\text{SO}_4$.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards (11)—

USP Penbutolol Sulfate RS

Identification, Ultraviolet Absorption (197U)—

Solution: Sonicate a weighed portion of ground Tablets in sufficient methanol to obtain a solution containing about 0.4 mg of penbutolol sulfate per mL. Filter this solution, and dilute a portion of the filtrate with methanol to obtain a solution containing about 0.06 mg of penbutolol sulfate per mL.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Mobile phase—Dissolve 2 g of ammonium acetate in 250 mL of water, add 750 mL of acetonitrile, mix, and adjust with glacial acetic acid to a pH of 6.0. Filter and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard solution—Dissolve an accurately weighed quantity of USP Penbutolol Sulfate RS quantitatively in water to obtain a stock solution having a known concentration of about 0.018 mg per mL. Mix 10.0 mL of this solution and

10.0 mL of acetonitrile, and filter through a filter having a 0.5- μ m or finer porosity.

Test solution—Filter about 30 mL of the solution under test. Mix 10.0 mL of the filtrate and 10.0 mL of acetonitrile, and filter through a filter having a 0.5- μ m or finer porosity.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 272-nm detector, a 4.6-mm \times 15-cm column that contains 5- μ m diameter packing L10. The flow rate is about 2.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.5%.

Procedure—Separately inject equal volumes (about 50 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, and measure the areas of the responses for the penbutolol peaks. Calculate the quantity, in mg, of $(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$ dissolved by the formula:

$$1800C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Penbutolol Sulfate RS in the *Standard solution*, and r_U and r_S are the penbutolol peak responses obtained from the *Test solution* and the *Standard solution*, respectively.

Tolerances—Not less than 75% (Q) of the labeled amount of $(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$ is dissolved in 30 minutes.

Chromatographic purity—Examine the chromatogram of the *Assay preparation* obtained in the *Assay*. If an impurity peak is observed at a retention time of 0.8 relative to that of penbutolol, calculate the percentage of that impurity by the formula:

$$100r_i / r_s$$

in which r_i is the response of the impurity peak, and r_s is the sum of the responses of all of the peaks. If the percentage exceeds 1.2%, perform the following test.

Organic phase, Aqueous phase, Solvent mixture, and Chromatographic system—Proceed as directed in the test for *Chromatographic purity* under *Penbutolol Sulfate*.

Test solution—Transfer an accurately weighed portion of powdered Tablets, equivalent to about 100 mg of penbutolol sulfate, to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, mix, and filter.

Diluted test solution—Transfer 1.0 mL of the *Test solution* to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the test for *Chromatographic purity* under *Penbutolol Sulfate*. Calculate the percentage of each individual impurity in the portion of Tablets taken by the formula:

$$r_i / r_D$$

in which the terms are as defined therein: not more than 1.2% of any impurity is found.

Assay—

Organic phase, Aqueous phase, and Mobile phase—Proceed as directed in the *Assay* under *Penbutolol Sulfate*.

Standard preparation—Dissolve an accurately weighed quantity of USP Penbutolol Sulfate RS quantitatively in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

Resolution solution—Prepare a solution of 3,4-dimethylbenzophenone in *Standard preparation* containing about 0.01 mg of 3,4-dimethylbenzophenone per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of penbutolol sulfate, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Sonicate for about 10 minutes, and filter a portion through a filter having a 0.5- μ m or finer

porosity, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 270-nm detector and a 4.6-mm \times 15-cm column that contains 5- μ m diameter packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for penbutolol and 1.0 for 3,4-dimethylbenzophenone, and the tailing factor is not more than 1.4, when calculated by the formula:

$$W_{0.1} / 2f$$

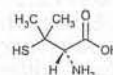
in which $W_{0.1}$ is the width of the peak at 10% of peak height, the resolution, R , between the penbutolol peak and the 3,4-dimethylbenzophenone peak is not less than 5, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$ in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Penbutolol Sulfate RS in the *Standard preparation*, and r_U and r_S are the penbutolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penicillamine



$C_5H_{11}NO_2S$ 149.21

D-Valine, 3-mercaptopropanoate.

D-3-Mercaptovaline [52-67-5].

» Penicillamine contains not less than 97.0 percent and not more than 102.0 percent of $C_5H_{11}NO_2S$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Penicillamine RS

USP Penicillin G Potassium RS

USP Penicillamine Disulfide RS

$C_{10}H_{20}N_2O_4S_2$

Identification—

A: Infrared Absorption (197M) (50 mg in 300 mg).

B: Dissolve 10 mg in 5 mL of water, and add 1 drop of 5 N sodium hydroxide and 20 mg of ninhydrin: a blue or violet-blue color is produced immediately.

C: Dissolve 20 mg in 4 mL of water, add 2 mL of phosphotungstic acid solution (1 in 10), and heat nearly to boiling: a deep blue color is produced immediately.

Specific rotation (781S): between -60.5° and -64.5° .

Test solution: 50 mg per mL, in 1.0 N sodium hydroxide.

pH (791): between 4.5 and 5.5, in a solution (1 in 100).

Loss on drying (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a

pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.1%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

Delete the following:

• **Heavy metals, Method II** (231): not more than 0.002%.

• (Official 1-Jan-2018)

Change to read:

Limit of penicillin activity—

pH 2.5 Buffer—Dissolve 100 g of monobasic potassium phosphate in water, add 0.2 mL of hydrochloric acid, dilute with water to 1000 mL, and mix. Adjust, if necessary, with phosphoric acid or with 10 N potassium hydroxide to a pH of 2.5.

Standard preparation—Prepare as directed for Penicillin G in Table 1 under *Antibiotics—Microbial Assays* (81), except to prepare a final stock solution containing 100 Penicillin G Units per mL and six test dilutions ranging from 0.005 Penicillin G Unit per mL to 0.2 Penicillin G Unit per mL, and to use a median dose of the Standard of 0.050 Penicillin G Unit per mL.

Test preparation—Dissolve 1.0 g in water to make 18.0 mL, transfer 9.0 mL of this solution to a separator, add 20 mL of amyl acetate and 1 mL of pH 2.5 Buffer, and shake. Allow the layers to separate, and draw off the aqueous layer into a second separator, retaining the amyl acetate extract in the first separator. Check the pH of the aqueous layer, and if it is greater than 3.0 adjust it with hydrochloric acid to a pH of 2.5, and extract with 20 mL of amyl acetate. Discard the aqueous layer, and add the amyl acetate extract to the first separator. Wash the combined amyl acetate extracts with 10 mL of diluted pH 2.5 Buffer (1 in 10), and discard the aqueous layer. Extract the amyl acetate with 10.0 mL of Buffer B.1 (see *Antibiotics—Microbial Assays* (81), *Media and Solutions, Solutions, Buffers*). • (CN 1-May-2017) Use a portion of the buffer extract as *Test solution A*. To a 5-mL portion of the extract add 0.1 mL of penicillinase solution, and incubate at 36° to 37.5° for 60 minutes (*Test solution B*).

Preparation of inoculum—Prepare as directed under *Antibiotics—Microbial Assays* (81), using *Micrococcus luteus* (ATCC 9341) as the test organism, and an inoculum that gives clear sharp zones of inhibition 17 mm to 21 mm in diameter with the median dose level of the Standard.

Procedure—Proceed as directed for the *Cylinder-Plate Method* under *Antibiotics—Microbial Assays* (81), using 10 mL of Medium 1 for the base layer and 4 mL of inoculated Medium 4 for the seed layer, and incubating the plates at 29° to 31°, except on each test plate to fill 2 cylinders with *Test solution A*, 2 cylinders with *Test solution B*, and 2 cylinders with the median dose of the Standard. If *Test solution A* yields no zone of inhibition, the test is negative for penicillin. If *Test solution A* yields a zone of inhibition and *Test solution B* does not, penicillin is present. Determine its level from the standard curve: not more than 0.01 Penicillin G Unit is found in each mL of *Test solution A* (0.2 Penicillin G Unit per g).

Mercury—

NOTE—Mercuric dithizonate is light-sensitive. Perform this test in subdued light.

Dithizone stock solution—Dissolve 40 mg of dithizone in 1000 mL of chloroform.

Dithizone titrant—Dilute 30.0 mL of *Dithizone stock solution* with chloroform to 100.0 mL. This solution contains approximately 12 mg of dithizone per L.

Standard solution—Transfer 135.4 mg of mercuric chloride to a 100-mL volumetric flask, add 0.25 N sulfuric acid to

volume, and mix. This solution contains the equivalent of 100 µg of Hg in 100 mL.

Diluted standard solution—Pipet 2 mL of *Standard solution* into a 100-mL volumetric flask, add 0.25 N sulfuric acid to volume, and mix. Each mL of this solution contains the equivalent of 20 µg of Hg.

Standardization—Pipet 1 mL of *Diluted standard solution* into a 250-mL separator, and add 100 mL of 0.25 N sulfuric acid, 90 mL of water, and 10 mL of hydroxylamine hydrochloride solution (1 in 5). Then add 1 mL of edetate disodium solution (1 in 50), 1 mL of glacial acetic acid, and 5 mL of chloroform, shake for 1 minute, allow to separate, and discard the chloroform layer. To the solution add *Dithizone titrant*, in portions of 0.3 mL to 0.5 mL, from a 10-mL buret. After each addition, shake the mixture 20 times, and allow the chloroform layer to separate and discard it. Continue until an addition of *Dithizone titrant* remains green after the shaking. Calculate the quantity, in µg, of mercury equivalent to 1 mL of *Dithizone titrant* by dividing 20 by the number of mL of *Dithizone titrant* added.

Procedure—Transfer 500 mg of Penicillamine to a 650-mL Kjeldahl flask containing a few glass beads, incline the flask at an angle of about 45°, and add 2.5 mL of nitric acid through a small funnel placed in the mouth of the flask. Allow the mixture to stand at room temperature until nitrous oxide fumes are evolved and vigorous reaction subsides (5 to 30 minutes). Add 2.5 mL of sulfuric acid through the funnel, and heat, gently at first and then to the production of fumes of sulfur trioxide, then cool. Cautiously add 2.5 mL of nitric acid, again heat to the production of sulfur trioxide fumes, and cool. Repeat the treatment with nitric acid and heat, then cool, and cautiously add 50 mL of water, rinsing the funnel and collecting the rinsings in the flask. Remove the funnel, boil the solution down to approximately half its volume (about 25 mL), and cool to room temperature. Transfer to a 250-mL separator with the aid of water, and add water to make about 50 mL. Add 1 mL of edetate disodium solution (1 in 50) and 1 mL of glacial acetic acid, and extract with small portions of chloroform until the last chloroform extract remains colorless. Discard the chloroform extract, and add 50 mL of 0.25 N sulfuric acid, 90 mL of water, and 10 mL of hydroxylamine hydrochloride solution (1 in 5). Add *Dithizone titrant*, in portions of 0.3 mL to 0.5 mL, from a 10-mL buret. After each addition, shake the mixture 20 times, and allow the chloroform layer to separate and discard it. Continue until an addition of *Dithizone titrant* remains green after the shaking. Calculate the amount of mercury present: the limit is 10 µg (0.002%).

Limit of penicillamine disulfide—

Diluent, Mobile phase, and Resolution solution—Prepare as directed in the Assay.

Standard preparation—Dissolve an accurately weighed quantity of USP Penicillamine Disulfide RS in *Diluent* to obtain a solution having a known concentration of about 0.025 mg per mL.

Test preparation—Use the Assay preparation.

Chromatographic system—Proceed as directed in the Assay. Chromatograph the *Standard preparation*, and record the penicillamine disulfide peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the penicillamine disulfide peaks. Calculate the percentage of penicillamine disulfide ($C_{10}H_{20}N_2O_4S_2$) in the Penicillamine taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S is the concentration, in mg per mL, of USP Penicillamine Disulfide RS in the *Standard preparation*, C_U is

the concentration, in mg per mL, of Penicillamine in the *Test preparation*, and r_U and r_S are the penicillamine disulfide peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 1.0% of penicillamine disulfide is found.

Assay—

Diluent—Dissolve 1.0 g of edetate disodium in water to make 1000 mL of solution.

Mobile phase—Dissolve 6.9 g of monobasic sodium phosphate and 0.20 g of sodium 1-hexanesulfonate in water to make 1000 mL of solution. Adjust with phosphoric acid to a pH of 3.0 ± 0.1 , and filter through a suitable filter of 1 μ m or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Resolution solution—Prepare a solution in *Diluent* containing about 1 mg of USP Penicillamine RS and 0.1 mg of USP Penicillamine Disulfide RS per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Penicillamine RS in *Diluent* to obtain a solution having a concentration of about 1.25 mg per mL.

Assay preparation—Transfer about 125 mg of Penicillamine, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 3.9-mm \times 30-cm column containing packing L1. The flow rate is about 1.6 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the relative retention times are about 0.7 for penicillamine and 1.0 for penicillamine disulfide, and the resolution, R , between the penicillamine peak and the penicillamine disulfide peak is not less than 3.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of penicillamine ($C_5H_{11}NO_2S$) in the portion of Penicillamine taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Penicillamine RS in the *Standard preparation*, and r_U and r_S are the penicillamine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penicillamine Capsules

» Penicillamine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_5H_{11}NO_2S$.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Penicillamine RS
USP Penicillamine Disulfide RS
 $C_{10}H_{20}N_2O_4S_2$

Identification—The contents of the Capsules respond to *Identification* test A under *Penicillamine Tablets* and to *Identification* test C under *Penicillamine*.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

PROCEDURE FOR A POOLED SAMPLE—

Dilute hydrochloric acid—Dilute 37 mL of hydrochloric acid with water to 1 L.

Ammonium sulfamate reagent—Dissolve 250 mg of ammonium sulfamate in 100 mL of *Dilute hydrochloric acid*.

***N*-(1-Naphthyl)ethylenediamine dihydrochloride reagent**—Dissolve 100 mg of *N*-(1-naphthyl)ethylenediamine dihydrochloride in 100 mL of *Dilute hydrochloric acid*.

Sulfanilamide-mercuric chloride reagent—Dissolve 100 mg of sulfanilamide and 100 mg of mercuric chloride in 100 mL of *Dilute hydrochloric acid*.

Sodium nitrite reagent—Dissolve 200 mg of sodium nitrite in 100 mL of dilute sulfuric acid (1 in 50). Prepare fresh.

Standard solution—Dissolve an accurately weighed quantity of USP Penicillamine RS in 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 250 μ g per mL.

Procedure—Pipet an aliquot of the filtered test solution, estimated to contain about 278 μ g of penicillamine, into a 100-mL volumetric flask. Into a similar flask pipet an equivalent volume of 0.1 N hydrochloric acid to provide a reagent blank, and into a third 100-mL volumetric flask pipet 1 mL of *Standard solution*. Treat each flask as follows. Add by pipet 3 mL of *Sodium nitrite reagent*, and mix by swirling occasionally. After 5 minutes, add 10 mL of *Ammonium sulfamate reagent*, swirl, and allow to stand for an additional 5 minutes. Add 5 mL of *Sulfanilamide-mercuric chloride reagent*, swirl, and immediately add 10 mL of *N*-(1-Naphthyl)ethylenediamine dihydrochloride reagent. Dilute with water to volume, and mix. Determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 540 nm, with a suitable spectrophotometer, against the reagent blank. Calculate the percentage dissolution of the Capsule taken by the formula:

$$90(C/WV)(A_U / A_S)$$

in which C is the concentration, in μ g per mL, of USP Penicillamine RS in the *Standard solution*; W is the labeled quantity, in mg, of penicillamine in the Capsule; V is the volume, in mL, of the aliquot of test solution used; and A_U and A_S are the absorbances of the solutions from the test solution and the *Standard solution*, respectively.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_5H_{11}NO_2S$ is dissolved in 30 minutes.

PROCEDURE FOR A UNIT SAMPLE —

Buffer solution—Prepare a 50-mM solution of monobasic potassium phosphate buffer, pH 3.0.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (97:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard solution—Prepare a solution of USP Penicillamine RS in 0.1 N hydrochloric acid having a known concentration corresponding to the content of 1 Capsule dissolved in 900 mL of *Medium*.

Resolution solution—Prepare a solution of USP Penicillamine Disulfide RS in 0.1 N hydrochloric acid having a known concentration of about 0.002 mg per mL.

Test solution—Proceed as directed for *Procedure* for *Capsules*, *Uncoated Tablets*, and *Plain Coated Tablets* under *Dissolution* (711). After 30 minutes, withdraw about 10 mL of solution from each vessel, and immediately pass each aliquot through a 0.45- μ m polyvinylidene difluoride filter paper. Discard the first 2 mL of filtered solution, and chromatograph the remaining filtrate.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 15-cm column that contains 5-μm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, R , between penicillamine and penicillamine disulfide is not less than 2.0.

Procedure—Separately inject equal volumes (about 30 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount, in percentage, of $C_5H_{11}NO_2S$ released by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times LC}$$

in which r_U and r_S are the peak areas obtained from the *Test solution* and the *Standard solution*, respectively; C_S is the concentration, in mg per mL, of the *Standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and LC is the label claim, in mg, for each Capsule.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_5H_{11}NO_2S$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Loss on drying (731)—Dry about 100 mg of Capsule contents, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 1.0% of its weight.

Limit of penicillamine disulfide—

Diluent, Mobile phase, and Resolution solution—Proceed as directed in the *Assay* under *Penicillamine*.

Standard preparation—Dissolve an accurately weighed quantity of USP Penicillamine Disulfide RS in *Diluent* to obtain a solution having a known concentration of about 0.025 mg per mL.

Test preparation—Use the *Assay preparation*.

Chromatographic system—Proceed as directed in the *Assay* under *Penicillamine*. Chromatograph the *Standard preparation*, and record the penicillamine disulfide peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the penicillamine disulfide peaks. Calculate the percentage of penicillamine disulfide ($C_{10}H_{20}N_2O_4S_2$) in the Capsules taken by the formula:

$$100(C/L)(V/N)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Penicillamine Disulfide RS in the *Standard preparation*; L is the quantity, in mg, of penicillamine in each Capsule based on the labeled amount; V is the volume, in mL, of the volumetric flask used to prepare the *Assay preparation* in the *Assay*; N is the number of Capsules used to prepare the *Assay preparation* in the *Assay*; and r_U and r_S are the penicillamine disulfide peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 2.0% of penicillamine disulfide is found.

Assay—

Diluent, Mobile phase, Resolution solution, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay* under *Penicillamine*.

Assay preparation—Carefully open and transfer the contents of not fewer than 10 Capsules, accurately counted, to a suitable volumetric flask of such volume that when treated as described below, a solution is obtained that contains about 1.25 mg of penicillamine per mL. Add the empty Capsule shells to the flask, and add sufficient *Diluent* to the flask to fill it to about three-fourths of its capacity. Shake for 1 minute, and allow the mixture to stand for 90 minutes. Dilute with *Diluent* to volume, and mix. Filter a portion of this solution through a suitable filter of 1 μm or finer porosity, and use the clear filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Penicillamine*. Calculate the quantity, in mg, of penicillamine ($C_5H_{11}NO_2S$) in each Capsule taken by the formula:

$$C(V/N)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Penicillamine RS in the *Standard preparation*; V is the volume, in mL, of the volumetric flask used to prepare the *Assay preparation*; N is the number of Capsules used to prepare the *Assay preparation*; and r_U and r_S are the penicillamine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penicillamine Tablets

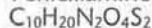
» Penicillamine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_5H_{11}NO_2S$.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Penicillamine RS

USP Penicillamine Disulfide RS



Identification—

A: Transfer a portion of finely powdered Tablets, equivalent to about 100 mg of penicillamine, to a 10-mL volumetric flask, dilute with methanol to volume, add 2 drops of 3 N hydrochloric acid, mix, and filter. Use the filtrate as the test solution. Prepare a *Standard solution* by dissolving 100 mg of USP Penicillamine RS in 10 mL of methanol, adding 2 drops of 3 N hydrochloric acid, and mixing. Separately apply 10-μL portions of the test solution and the *Standard solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture, heated at 105° for 30 minutes, and allowed to cool before use. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of butyl alcohol, glacial acetic acid, and water (8:2:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, mark the solvent front, allow the solvent to evaporate, and place the plate in an atmosphere of iodine vapors. After a few minutes, spray the plate with a 1 in 300 solution of ninhydrin in dehydrated alcohol, heat it at 105° for about 10 minutes, allow it to cool, and examine it: the R_f values, colors, and intensities of the principal spots obtained from the test solution correspond to those obtained from the *Standard solution*.

B: A portion of powdered Tablets responds to *Identification test C* under *Penicillamine*.

Dissolution (711)—

Medium: 0.5% edetate disodium and 0.05% sodium lauryl sulfate solution; 900 mL.

Apparatus 1: 100 rpm.

Time: 60 minutes.

Mobile phase—Prepare a filtered and degassed solution of 0.01 M dibasic sodium phosphate and 0.01 M monobasic potassium phosphate (60:40). If necessary, adjust the solution by the addition of 0.01 M dibasic sodium phosphate or 0.01 M monobasic potassium phosphate to a pH of 7.0 ± 0.1 .

Standard solution—Prepare a solution of USP Penicillamine RS in 0.5% edetate disodium and 0.05% sodium lauryl sulfate solution having an accurately known concentration of about 0.28 mg per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm \times 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%, and the resolution factor between the solvent peak and penicillamine is not less than 1.5.

Procedure—Separately inject equal volumes (about 80 μ L) of the *Standard solution* and a filtered portion of the solution under test into the chromatograph, record the chromatograms, measure the responses for the major peaks, and calculate the amount of $C_5H_{11}NO_2S$ dissolved per Tablet.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_5H_{11}NO_2S$ is dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements.

Loss on drying (731)—Dry about 100 mg of finely ground Tablets, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 3.0% of its weight.

Penicillamine disulfide—

Diluent—Prepare as directed in the *Assay*.

Mobile phase, Resolution solution, and Chromatographic system—Proceed as directed in the *Assay* under *Penicillamine*.

Standard preparation—Dissolve an accurately weighed quantity of USP Penicillamine Disulfide RS in *Diluent* to obtain a solution having a known concentration of about 0.025 mg per mL.

Test preparation—Use the *Assay preparation*.

Chromatographic system—Proceed as directed in the *Assay* under *Penicillamine*. Chromatograph the *Standard preparation*, and record the penicillamine disulfide peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the penicillamine disulfide peaks. Calculate the percentage of penicillamine disulfide ($C_{10}H_{20}N_2O_4S_2$) in the portion of Tablets taken by the formula:

$$20,000(C/L)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Penicillamine Disulfide RS in the *Standard preparation*, L is the quantity, in mg, of penicillamine in each Tablet based on the labeled amount, and r_U and r_S are the penicillamine disulfide peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 3.0% of penicillamine disulfide is found.

Assay—

Diluent—Dissolve 5.0 g of edetate disodium in water to make 1000 mL of solution.

Mobile phase, Resolution solution, and Chromatographic system—Proceed as directed in the *Assay* under *Penicillamine*.

Standard preparation—Dissolve an accurately weighed quantity of USP Penicillamine RS in *Diluent* to obtain a solution having a known concentration of about 1.25 mg per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of penicillamine, to a 200-mL volumetric flask, add about 150 mL of *Diluent*, shake for 5 minutes, and allow the mixture to stand for 90 minutes. Dilute with *Diluent* to volume, and mix. Filter a portion of this solution through a suitable filter of 1 μ m or finer porosity, and use the clear filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Penicillamine*. Calculate the quantity, in mg, of penicillamine ($C_5H_{11}NO_2S$) in the portion of Tablets taken by the formula:

$$200C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Penicillamine RS in the *Standard preparation*, and r_U and r_S are the penicillamine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penicillin G, Neomycin, Polymyxin B, Hydrocortisone Acetate, and Hydrocortisone Sodium Succinate Topical Suspension

» Penicillin G, Neomycin, Polymyxin B, Hydrocortisone Acetate, and Hydrocortisone Sodium Succinate Topical Suspension is a suspension of Penicillin G Procaine, Neomycin Sulfate, Polymyxin B Sulfate, Hydrocortisone Acetate, and Hydrocortisone Sodium Succinate in a suitable vehicle. It contains not less than 90.0 percent and not more than 140.0 percent of the labeled amounts of neomycin, penicillin G, and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of hydrocortisone acetate ($C_{23}H_{32}O_6$) and hydrocortisone sodium succinate ($C_{25}H_{33}NaO_8$).

Packaging and storage—Preserve in well-closed containers.

Labeling—Label Topical Suspension to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Hydrocortisone Acetate RS
USP Hydrocortisone Hemisuccinate RS
USP Neomycin Sulfate RS
USP Penicillin G Potassium RS
USP Polymyxin B Sulfate RS

Identification—

A: Shake a quantity of Topical Suspension, equivalent to about 50,000 USP Polymyxin B Units, with 20 mL of chloroform, add 5 mL of 0.1 N hydrochloric acid, shake vigorously, centrifuge, and use the clear supernatant liquid as the *Sample solution*. Apply separately 10 μ L of the *Sample solution* and 10 μ L of a *Standard solution* of USP Polymyxin B Sulfate RS in 0.1 N hydrochloric acid containing 10,000 USP Polymyxin B Units per mL to a suitable thin-layer chromatographic plate.

graphic plate coated with a 0.25-mm layer of dimethylsilanized chromatographic silica gel mixture (see *Chromatography* (621)). Place the plate in a suitable chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of isopropyl alcohol, water, and ammonium hydroxide (24:17:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 105° for 5 minutes. Spray the plate with a 1 in 200 solution of ninhydrin in butyl alcohol, and heat the plate at 105° for 15 minutes: the R_f value of the principal spot in the chromatogram obtained from the *Sample solution* corresponds to that of the principal spot in the chromatogram obtained from the *Standard solution*.

B: It responds to the Identification test under *Penicillin G Procaine Intramammary Infusion*.

C: The chromatogram of the *Assay preparation* obtained as directed in the *Assay for hydrocortisone acetate and hydrocortisone sodium succinate* exhibits major peaks for hydrocortisone acetate and hydrocortisone sodium succinate, the retention times of which, relative to that of the internal standard, correspond to those for hydrocortisone acetate and hydrocortisone hemisuccinate exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay for hydrocortisone acetate and hydrocortisone sodium succinate*.

Water Determination, Method I (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Assay for penicillin G—Proceed with the Topical Suspension as directed in the *Assay for penicillin G under Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension*.

Assay for neomycin—Proceed with the Topical Suspension as directed in the *Assay for neomycin under Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension*.

Assay for polymyxin B—Proceed with the Topical Suspension as directed in the *Assay for polymyxin B under Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension*.

Assay for hydrocortisone acetate and hydrocortisone sodium succinate—

Mobile phase—Prepare a filtered and degassed mixture of butyl chloride, water-saturated butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (544:544:58:29:25). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Extraction solution—Prepare a mixture of chloroform and glacial acetic acid (1000:30).

Internal standard solution—Prepare a solution in tetrahydrofuran containing about 1.4 mg of methylprednisolone per mL.

Standard preparation—Transfer about 7.5 mg of USP Hydrocortisone Acetate RS, accurately weighed, and 7.5J mg of USP Hydrocortisone Hemisuccinate RS, accurately weighed, to a conical flask, J being the ratio of the labeled amount, in mg, of hydrocortisone sodium succinate to the labeled amount, in mg, of hydrocortisone acetate in the Topical Suspension. Add 5.0 mL of *Internal standard solution* and about 95 mL of *Extraction solution*, and mix.

Resolution solution—Dissolve about 3.7 mg of penicillin G procaine in 10 mL of *Standard preparation*.

Assay preparation—Transfer an accurately measured portion of well-mixed Topical Suspension, equivalent to about 7.5 mg of hydrocortisone acetate, to a conical flask. Add 5.0 mL of *Internal standard solution* and about 95 mL of *Extraction solution*, and shake by mechanical means for about 15 minutes. Centrifuge a portion of this mixture, and use the clear supernatant as the *Assay preparation*.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm de-

tector, a guard column containing packing L3, and a 3.9-mm × 30-cm analytical column that contains packing L3. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and measure the peak responses as directed for *Procedure*: the relative retention times for penicillin G procaine, hydrocortisone acetate, hydrocortisone hemisuccinate, and methylprednisone are about 0.3, 0.4, 0.7, and 1.0, respectively, and the resolution, R_s , between penicillin G procaine and hydrocortisone acetate is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation of the ratios of the hydrocortisone acetate peak to the internal standard peak and the hydrocortisone sodium succinate peak to the internal standard peak is not more than 2%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of hydrocortisone acetate in the portion of Topical Suspension taken by the formula:

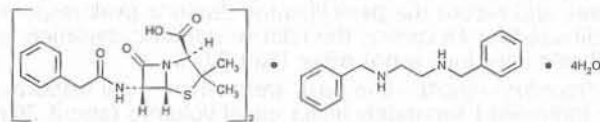
$$W(R_U / R_S)$$

in which W is the quantity, in mg, of USP Hydrocortisone Acetate RS taken to prepare the *Standard preparation*, and R_U and R_S are the ratios of the hydrocortisone acetate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of hydrocortisone sodium succinate in the portion of Topical Suspension taken by the formula:

$$(484.52 / 462.54)(W)(R_U / R_S)$$

in which 484.52 and 462.54 are the molecular weights of hydrocortisone sodium succinate and anhydrous hydrocortisone hemisuccinate, respectively, W is the quantity, in mg, of USP Hydrocortisone Hemisuccinate RS taken to prepare the *Standard preparation*, and R_U and R_S are the ratios of the hydrocortisone hemisuccinate peak response to the internal standard peak response obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penicillin G Benzathine



($C_{16}H_{18}N_2O_4S$)₂ · $C_{16}H_{20}N_2$ · 4H₂O 981.18
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-, 2[5-(2α,5α,6β)-, compd. with *N,N'*-bis(phenylmethyl)-1,2-ethanediamine (2:1), tetrahydrate.
(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with *N,N'*-dibenzylethylenediamine (2:1), tetrahydrate [41372-02-5].
Anhydrous 909.15 [1538-09-6].

» Penicillin G Benzathine has a potency of not less than 1090 Penicillin G Units and not more than 1272 Penicillin G Units per mg.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must

be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards (11)—

USP Endotoxin RS
USP Penicillin G Benzathine RS
USP Penicillin G Potassium RS

Identification, *Ultraviolet Absorption* (197U)—

Solution: 500 µg per mL.

Medium: methanol.

Absorptivity at 263 nm is between 85.0% and 110.0% of that of USP Penicillin G Benzathine RS.

Crystallinity (695): meets the requirements.

Bacterial Endotoxins Test (85)—Where the label states that Penicillin G Benzathine is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms it contains not more than 0.01 USP Endotoxin Unit per 100 Penicillin G Units.

Sterility Tests (71)—Where the label states that Penicillin G Benzathine is sterile it meets the requirements when tested as directed in the section *Direct Inoculation of the Culture Medium under Test for Sterility of the Product to be Examined*, except to use Fluid Thioglycollate Medium and Soybean-Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the penicillin G in each tube, and to shake the vessels once daily.

pH (791): between 4.0 and 6.5, in a solution prepared by dissolving 50 mg in 50 mL of dehydrated alcohol, adding 50 mL of water, and mixing.

Water Determination, *Method I* (921): between 5.0% and 8.0%.

Benzathine content—To about 1 g of Penicillin G Benzathine, accurately weighed, add 30 mL of a saturated solution of sodium chloride and 10 mL of 5 N sodium hydroxide, and extract with four 50-mL portions of ether. Wash the combined ether extracts with three 10-mL portions of water. Extract the combined water washings with 25 mL of ether, and add the ether extract to the water-washed combined ether extracts. Evaporate this combined ether solution to a volume of about 5 mL, add 2 mL of dehydrated alcohol, and evaporate to dryness. Dissolve the residue in 50 mL of glacial acetic acid, add 1 mL of *p*-naphtholbenzene TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 12.02 mg of benzathine ($C_{16}H_{20}N_2$): between 24.0% and 27.0% of benzathine in Penicillin G Benzathine, calculated on the anhydrous basis, is found.

Assay—

0.05 M phosphate buffer, pH 6.0—Dissolve 6.8 g of monobasic potassium phosphate in 900 mL of water, adjust with 1 N sodium hydroxide to a pH of 6.0, dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a mixture of *0.05 M phosphate buffer, pH 6.0* and acetonitrile (4:1), pass through a membrane filter having a 5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Transfer about 40 mg of USP Penicillin G Potassium RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of acetonitrile and 5 mL of methanol, and swirl to dissolve. Without delay, dilute with *0.05 M phosphate buffer, pH 6.0* to volume, and mix.

System suitability preparation—Prepare a solution of penicillin V potassium in *Mobile phase* containing about 1 mg per mL. Mix equal volumes of this solution and the *Standard preparation*.

Assay preparation—Transfer about 53 mg of Penicillin G Benzathine, accurately weighed, to a 50-mL volumetric flask, add 10 mL of acetonitrile and 5 mL of methanol, and swirl

to dissolve. Without delay, dilute with *0.05 M phosphate buffer, pH 6.0* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 225-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for penicillin G and 1.0 for penicillin V; the resolution, *R*, between penicillin G and penicillin V is not less than 2.0; the column efficiency determined from the analyte peak is not less than 600 theoretical plates; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the potency, in Penicillin G Units per mg, of the Penicillin G Benzathine taken by the formula:

$$50(CP/W)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Penicillin G Potassium RS in the *Standard preparation*; *P* is the stated potency, in Penicillin G Units per mg, of USP Penicillin G Potassium RS; *W* is the quantity, in mg, of Penicillin G Benzathine taken to prepare the *Assay preparation*; and *r_U* and *r_S* are the penicillin G peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penicillin G Benzathine Injectable Suspension

» Penicillin G Benzathine Injectable Suspension is a sterile suspension of Penicillin G Benzathine in Water for Injection with one or more suitable buffers, dispersants, preservatives, and suspending agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of penicillin.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass, in a refrigerator.

USP Reference standards (11)—

USP Endotoxin RS
USP Penicillin G Benzathine RS
USP Penicillin G Potassium RS

Identification—It responds to the *Identification* test under *Penicillin G Benzathine Oral Suspension*.

Bacterial Endotoxins Test (85)—It contains not more than 0.01 Endotoxin Unit per 100 Penicillin G Units.

Sterility Tests (71)—It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium under Test for Sterility of the Product to be Examined*, except to use Fluid Thioglycollate Medium and Soybean-Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the penicillin G in each vessel, and to shake the vessels once daily.

pH (791): between 5.0 and 7.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Change to read:**Assay—**

Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* (425).

Assay preparation—Using a suitable hypodermic needle and syringe, withdraw an accurately measured volume of Injectable Suspension, equivalent to about 300,000 Penicillin G Units, and dilute quantitatively with 1.0 N sodium hydroxide to obtain an *Assay preparation* containing about 2000 Penicillin G Units per mL. Pipet 2.0 mL of this solution into a glass-stoppered, 125-mL conical flask.

Blank preparation—Using a suitable hypodermic needle and syringe, withdraw an accurately measured volume of Injectable Suspension, equivalent to about 300,000 Penicillin G Units, and quantitatively dilute with *Buffer B.1* (CN 1-May-2017) to obtain a suspension containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425), except in performing the *In-activation and Titration* to omit the addition of 1.0 N sodium hydroxide to the *Assay preparation*, and in performing the *Blank Determination* to use the *Blank preparation* in place of the *Assay preparation*. Calculate the quantity, in Penicillin G Units, in each mL of the Injectable Suspension taken by the formula:

$$(L / 2D)(F(B - I))$$

in which *L* is the labeled quantity, in Penicillin G Units per mL, in the Injectable Suspension taken, and *D* is the concentration, in Penicillin G Units per mL, in the *Assay preparation* on the basis of the labeled quantity in the Injectable Suspension and the extent of dilution, and the other terms are as defined therein.

Penicillin G Benzathine Oral Suspension

» Penicillin G Benzathine Oral Suspension contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of penicillin G. It contains one or more suitable buffers, colors, dispersants, flavors, and preservatives.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Penicillin G Benzathine RS
USP Penicillin G Potassium RS

Identification—Mix a portion of it with methanol to obtain a solution containing about 3000 Penicillin G Units per mL. Apply 20 μ L of this test solution and 20 μ L of a Standard solution of USP Penicillin G Benzathine RS in methanol containing 2.5 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel, and allow the spots to dry. Using an unlined developing chamber, develop the chromatogram in a solvent system consisting of a mixture of methanol, acetonitrile, and ammonium hydroxide (70:30:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and allow to air-dry. Spray the plate uniformly with a spray reagent prepared as follows. Dissolve 20 g of tartaric acid and 1.7 g of bismuth subnitrate in 80 mL of water. Add 2.5 mL of this solution, 2.5 mL of potassium iodide solution (4 in 10), and 10 g of tartaric

acid to 50 mL of water, and mix. Examine the chromatograms: the principal spot obtained from the test solution corresponds in *R_f* value to that obtained from the Standard solution.

Uniformity of dosage units (905)—

FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698): meets the requirements.

pH (791): between 6.0 and 7.0.

Change to read:**Assay—**

Standard preparation—Using Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* (425).

Assay preparation—Dilute an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, quantitatively with 1.0 N sodium hydroxide to obtain a solution having a concentration of about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask.

Blank preparation—Dilute an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, quantitatively with *Buffer B.1* (CN 1-May-2017) to obtain a suspension containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425), except in performing the *In-activation and titration* to omit the addition of 1.0 N sodium hydroxide to the *Assay preparation*, and in performing the *Blank determination* to use the *Blank preparation* in place of the *Assay preparation*. Calculate the quantity, in Penicillin G Units, in each mL of the Oral Suspension taken by the formula:

$$(L / 2D)(F(B - I))$$

in which *L* is the labeled quantity, in Penicillin G Units per mL, in the Oral Suspension taken, and *D* is the concentration, in Penicillin G Units per mL, of the *Assay preparation* on the basis of the labeled quantity in the Oral Suspension and the extent of dilution.

Penicillin G Benzathine Tablets

» Penicillin G Benzathine Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of penicillin G.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Penicillin G Benzathine RS
USP Penicillin G Potassium RS

Identification—Shake a suitable quantity of finely powdered Tablets with methanol to obtain a solution containing about 3000 Penicillin G Units per mL, and filter: the filtrate so obtained responds to the *Identification* test under *Penicillin G Benzathine Oral Suspension*.

Disintegration (701): 60 minutes, simulated gastric fluid TS being used in place of water as the test medium.

Uniformity of dosage units (905): meet the requirements.

Water Determination, Method I (921): not more than 8.0%.

Change to read:**Assay—**

Standard preparation—Using Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* (425).

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer a portion of the powder, accurately weighed, equivalent to about 200,000 Penicillin G Units, to a 100-mL volumetric flask, add 10 mL of 1.0 N sodium hydroxide, and mix. Allow to stand for 15 minutes, add 10 mL of 1.2 N hydrochloric acid, dilute with water to volume, and mix. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask.

Blank preparation—Transfer an accurately weighed portion of the powdered Tablets remaining from the preparation of the *Assay preparation*, equivalent to about 200,000 Penicillin G Units, to a 100-mL volumetric flask, dilute with **Buffer B.1** (CN 1-May-2017) to volume, and mix. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425), except in performing the *Inactivation and titration* to omit the addition of 1.0 N sodium hydroxide and 1.2 N hydrochloric acid, and in the *Blank determination* to use the *Blank preparation* in place of the *Assay preparation*. Calculate the quantity, in Penicillin G Units, in the portion of Tablets taken by the formula:

$$(T/2D)(F(B - I))$$

in which *T* is the labeled quantity, in Penicillin G Units, in each Tablet, and *D* is the concentration, in Penicillin G Units per mL, of the *Assay preparation* on the basis of the labeled quantity in each Tablet and the extent of dilution.

Penicillin G Benzathine and Penicillin G Procaine Injectable Suspension

» Penicillin G Benzathine and Penicillin G Procaine Injectable Suspension is a sterile suspension of Penicillin G Benzathine and Penicillin G Procaine or, when labeled for veterinary use only, of Penicillin G Benzathine and penicillin G procaine, in Water for Injection. It may contain one or more suitable buffers, preservatives, and suspending agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of penicillin G benzathine and penicillin G procaine.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass.

Labeling—Where it is intended for veterinary use only, it is so labeled.

USP Reference standards (11)—

USP Endotoxin RS
USP Penicillin G Benzathine RS
USP Penicillin G Potassium RS
USP Procaine Hydrochloride RS

Identification—

A: It responds to the *Identification* test under *Penicillin G Benzathine Oral Suspension*: the spot obtained from the test solution, corresponding in *R_f* value to that obtained from the Standard solution, is completely resolved from a second spot, produced by penicillin G procaine.

B: It responds to the *Identification* test under *Penicillin G Procaine*.

Crystallinity (695) (where it is prepared from penicillin G procaine and is labeled as intended for veterinary use only)—Dilute a portion of the Injectable Suspension, equivalent to about 300,000 Penicillin G Units, with water to obtain a volume of 10 mL, and centrifuge. Remove and discard the supernatant fluid. Resuspend the residue in 10 mL of water, and centrifuge. Remove and discard the supernatant fluid. Dry the residue in a vacuum desiccator. The dried residue meets the requirements.

pH (791): between 5.0 and 7.5.

Limit of soluble penicillin G and procaine (where it is prepared from penicillin G procaine and is labeled for veterinary use only)—

Mobile phase—Dissolve 4 g of sodium 1-hexanesulfonate and 5.44 g of monobasic potassium phosphate in 760 mL of water, adjust with phosphoric acid to a pH of 2.5, dilute with acetonitrile to 1000 mL, and mix. Pass through a filter having a 0.5-μm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

pH 6.0 Phosphate buffer—Dissolve 16 g of monobasic potassium phosphate and 4 g of dibasic sodium phosphate in water, dilute with water to 200 mL, adjust with phosphoric acid or 1 N sodium hydroxide to a pH of 6.0.

Diluent—Transfer 60 mL of butyl alcohol, 100 mL of acetonitrile, and 10 mL of pH 6.0 Phosphate buffer to a 500-mL volumetric flask, dilute with water to volume, and mix.

Standard solution—Transfer about 24 mg of USP Penicillin G Potassium RS, accurately weighed, and about 8 mg of USP Procaine Hydrochloride RS, accurately weighed, to a 100-mL volumetric flask, add 12 mL of butyl alcohol and 20 mL of acetonitrile, and shake to dissolve. Add 2 mL of pH 6.0 Phosphate buffer and mix and bring to volume with water.

Test solution—Centrifuge about 20 mL of the Suspension. Remove the supernatant fluid, and pass it through a filter having a 5-μm or finer porosity. Transfer 5.0 mL of the clear filtrate to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 204-nm detector and a 4-mm × 15-cm column that contains 5-μm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 0.3%.

Procedure—Separately inject equal volumes (about 5 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of penicillin G (C₁₆H₁₈N₂O₄S) in the *Test solution* by the formula:

$$(334.40 / 372.48)(C)(r_U / r_S)$$

in which 334.40 and 372.48 are the molecular weights of penicillin G and penicillin G potassium respectively; *C* is the concentration, in mg per mL, of USP Penicillin G Potassium RS in the *Standard solution*; and *r_U* and *r_S* are the responses of the penicillin G peaks in the chromatograms of the *Test solution* and the *Standard solution*, respectively: not more than 1% is found. Calculate the percentage of procaine (C₁₃H₂₀N₂O₂) in the *Test solution* by the formula:

$$(236.32 / 272.78)(C)(r_U / r_S)$$

in which 236.32 and 272.78 are the molecular weights of procaine and procaine hydrochloride, respectively; *C* is the concentration, in mg per mL, of USP Procaine Hydrochloride RS in the *Standard solution*; and *r_U* and *r_S* are the responses of the procaine peaks in the chromatograms of the *Test so-*

lution and the *Standard solution*, respectively: not more than 1% is found.

Other requirements—It meets the requirements for *Bacterial endotoxins and Sterility* under *Penicillin G Procaine Injectable Suspension*. It meets also the requirements under *Injections and Implanted Drug Products* (1).

Assay for penicillin G procaine—

Standard preparations—Transfer about 14k mg of USP Procaine Hydrochloride RS, accurately weighed, to a 500-mL volumetric flask, and dissolve in 2 mL of 0.5 N sodium hydroxide, *k* being the ratio of the labeled number of Penicillin G Procaine Units to the labeled number of Penicillin G Benzathine Units in the Injectable Suspension. After 15 minutes, add 1 mL of 1.2 N hydrochloric acid, dilute with water to volume, and mix. This stock solution contains about 28k µg of USP Procaine Hydrochloride RS per mL. Transfer 1.0, 2.0, 3.0, 4.0, and 5.0 mL, respectively, of this stock solution to each of five 25-mL volumetric flasks. Transfer 4.0, 3.0, 2.0, and 1.0 mL of water to the first four flasks, respectively.

Assay preparation—Where the Injectable Suspension is represented as being in a single-dose container, withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe. Where the label states the quantities of penicillin G benzathine and penicillin G procaine in a given volume of Injectable Suspension, remove an accurately measured volume of the Injectable Suspension. For each 300,000 Penicillin G Benzathine Units in the specimen of Injectable Suspension taken, add 20 mL of 0.5 N sodium hydroxide, and mix. After 15 minutes, add 0.5 mL of 1.2 N hydrochloric acid for each mL of 0.5 N sodium hydroxide used, and dilute quantitatively with water to obtain a solution containing 36 Penicillin G Procaine Units per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask.

Procedure—To each of the flasks containing the *Standard preparations* and the *Assay preparation*, and to a seventh 50-mL volumetric flask containing 5.0 mL of water to provide the blank, add 0.5 mL of 4 N hydrochloric acid, 1.0 mL of sodium nitrite solution (1 in 1000), 1.0 mL of ammonium sulfamate solution (1 in 200), and 1.0 mL of N-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000), mixing and allowing 2 minutes to elapse after each addition. Dilute the contents of each flask with water to volume, and mix. Concomitantly determine the absorbances of the solutions from the *Standard preparations* and the *Assay preparation* at the wavelength of maximum absorbance at about 550 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. Plot the absorbance values of solutions from the *Standard preparations* versus concentration, in mg per mL, of procaine hydrochloride in the solutions from the *Standard preparations*, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration (*C*), in mg per mL, of procaine hydrochloride in the solution from the *Assay preparation*. Calculate the quantity, in Penicillin G Procaine Units in each container or in each mL of the Injectable Suspension taken by the formula:

$$(588.73 / 272.78)(1009.1)(CL / D)$$

in which 588.73 and 272.78 are the molecular weights of penicillin G procaine monohydrate and procaine hydrochloride, respectively; 1009.1 is the theoretical potency, in Penicillin G Units, in each mg of penicillin G procaine; *L* is the labeled amount, in Penicillin G Procaine Units in each container or in each mL of Injectable Suspension taken; and *D* is the concentration, in Penicillin G Procaine Units per mL, of the solution from the *Assay preparation*, on the basis of the labeled amount in each container or in each mL of Injectable Suspension taken and the extent of dilution.

Change to read:

Assay for penicillin G benzathine—

Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* (425).

Assay preparation—Where the Injectable Suspension is represented as being in a single-dose container, withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe. Where the label states the quantities of penicillin G benzathine and penicillin G procaine in a given volume of Injectable Suspension, remove an accurately measured volume of Injectable Suspension, freshly mixed but free from air bubbles. Dilute the specimen of Injectable Suspension taken quantitatively with 1 N sodium hydroxide to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Blank preparation—Prepare as directed for *Assay preparation*, except to use *Buffer B.1* (CN 1-May-2017) instead of 1.0 N sodium hydroxide.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425), except in performing the *Inactivation and Titration* to omit the addition of 1.0 N sodium hydroxide to the *Assay preparation*, and in performing the *Blank Determination* to use the *Blank preparation* in place of the *Assay preparation*. Calculate the total quantity, *T*, in Penicillin G Units, in each mL of the Injectable Suspension taken by the formula:

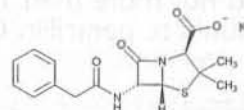
$$(L / 2D)(F)(B - I)$$

in which *L* is the labeled quantity, in Penicillin G Units in each container, or per mL, in the Injectable Suspension taken; and *D* is the concentration, in Penicillin G Units per mL, in the *Assay preparation* on the basis of the labeled quantity in the Injectable Suspension and the extent of dilution. Calculate the quantity, in Penicillin G Benzathine Units, in each container, or in each mL, of the Injectable Suspension taken by the formula:

$$T - P$$

in which *P* is the quantity, in Penicillin G Procaine Units, in each container, or in each mL, of Injectable Suspension taken, as determined in the *Assay for penicillin G procaine*.

Penicillin G Potassium



$C_{16}H_{17}KN_2O_4S$ 372.48
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-, monopotassium salt, [2S-(2 α ,5 α ,6 β)]-;
Monopotassium (2S,5R,6R)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [113-98-4].

DEFINITION

Penicillin G Potassium has a potency of NLT 1440 and NMT 1680 Penicillin G Units/mg.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
 - **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
 - **C.**
 - Diluent:** Glycerin and water (25:14)
 - Solution A:** 106 mg/mL of sodium carbonate in water
 - Solution B:** 120 mg/mL of sodium sulfide in *Diluent*, prepared as follows. Dissolve sodium sulfide in *Diluent*, using about 45% of the final volume and heat. Allow to cool, and dilute with *Diluent* to the final volume.
 - Solution C:** 150 mg/mL of tartaric acid in water
 - Sample solution:** 0.1 g of Penicillin G Potassium in 2 mL of water
- Analysis**
- Part 1:** Add 1 mL of *Solution A* to the *Sample solution* and heat.
- Part 2:** To the hot solution from *Part 1* add 0.05 mL of *Solution B*.
- Part 3:** Cool the mixture from *Part 2* in iced water and add 2 mL of *Solution C*. Allow to stand.
- Acceptance criteria:** Meets the requirements for *Parts 1, 2, and 3*
- Part 1:** No precipitate is formed.
- Part 2:** No precipitate is formed.
- Part 3:** A white precipitate is formed.

ASSAY• **PROCEDURE**

Solution A: 0.01 M monobasic potassium phosphate

Mobile phase: Methanol and *Solution A* (40:60)

System suitability solution: 0.1 mg/mL each of USP Penicillin G Potassium RS and 2-phenylacetamide in water

Standard solution: 0.1 mg/mL of USP Penicillin G Potassium RS in water. This solution contains about 160 Penicillin G Units/mL. Shake as needed to dissolve.

Sample solution: 0.1 mg/mL of Penicillin G Potassium in water. Shake as needed to dissolve.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 2-phenylacetamide and penicillin G are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between 2-phenylacetamide and penicillin G, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the potency of penicillin G potassium, in Penicillin G Units/mg, in the portion of Penicillin G Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response of penicillin G from the *Sample solution*

r_S = peak response of penicillin G from the *Standard solution*

C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin G Potassium in the *Sample solution* (mg/mL)

P = potency of USP Penicillin G Potassium RS (Penicillin G Units/mg)

Acceptance criteria: 1440–1680 Penicillin G Units/mg

SPECIFIC TESTS

- **CRYSTALLINITY (695):** Meets the requirements
- **pH (791)**
 - Sample solution:** 60 mg/mL of Penicillin G Potassium in water
 - Acceptance criteria:** 5.0–7.5
- **LOSS ON DRYING (731)**
 - Sample:** 100 mg of Penicillin G Potassium
 - Analysis:** Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.
 - Acceptance criteria:** NMT 1.5%
- **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Penicillin G Potassium is sterile or it must be subjected to further processing during the preparation of injectable dosage forms, it has NMT 0.01 USP Endotoxin Units/100 Penicillin G Units.
- **STERILITY TESTS (71):** Where the label states that Penicillin G Potassium is sterile, it meets the requirements.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS (11)**
 - USP Endotoxin RS
 - USP Penicillin G Potassium RS

Penicillin G Potassium Injection**DEFINITION**

Penicillin G Potassium Injection is a sterile isoosmotic solution of Penicillin G Potassium in Water for Injection. It contains one or more suitable buffers and a tonicity-adjusting agent. It contains NLT 90.0% and NMT 115.0% of the labeled number of Penicillin G Units.

IDENTIFICATION

- **A.** The retention time of the penicillin G peak of *Sample solution 1* or *Sample solution 2* corresponds to that of *Standard solution 2*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Buffer A: Dissolve 0.8 g of sodium citrate dihydrate in 150 mL of water. Adjust with 0.1 N hydrochloric acid to a pH of 6.8, and dilute with water to 200 mL.

Buffer B: Dissolve 10 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 4.15, and dilute with water to 1000 mL.

Mobile phase: Methanol and *Buffer B* (450:550)

Standard stock solution: 2000 Penicillin G Units/mL from USP Penicillin G Potassium RS in *Buffer A*

Standard solution 1: 100 Penicillin G Units/mL from *Standard stock solution* in water

Standard solution 2: 200 Penicillin G Units/mL from *Standard stock solution* in water

Standard solution 3: 300 Penicillin G Units/mL from *Standard stock solution* in water

Sample solution 1 (where it is represented as being in a single-dose container): Allow 1 container of Injection to thaw, and mix. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with water to obtain a solution containing nominally about 200 Penicillin G Units/mL.

Sample solution 2 (where the label states the quantity of penicillin G in a given volume of Injection): Allow 1

container of Injection to thaw, and mix. Dilute a suitable aliquot of the Injection with water to obtain a solution containing nominally about 200 Penicillin G Units/mL.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 10-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution 2*

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and *Sample solution 1* or *Sample solution 2*

Plot the peak responses from the *Standard solutions* versus concentration in Penicillin G Units/mL and draw the straight line best fitting the three plotted points. Calculate the percentage of the labeled number of Penicillin G Units in the portion of Injection taken:

$$\text{Result} = [(N \times D)/L] \times 100$$

- N* = concentration of *Sample solution 1* or *Sample solution 2* determined from the graph (Penicillin G Units/mL)
- D* = dilution factor for *Sample solution 1* or *Sample solution 2* (mL/mL)
- L* = labeled number of Penicillin G Units in Injection (Penicillin G Units/mL)

Acceptance criteria: 90.0%–115.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 0.01 USP Endotoxin Units/100 Penicillin G Units.
- **STERILITY TESTS** (71): It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **pH** (791): 5.5–8.0
- **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, as described in *Packaging and Storage Requirements* (659). Maintain in the frozen state.
- **LABELING:** It meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just before use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
- **USP REFERENCE STANDARDS** (11)
 - USP Endotoxin RS
 - USP Penicillin G Potassium RS

Penicillin G Potassium for Injection

DEFINITION

Penicillin G Potassium for Injection is sterile Penicillin G Potassium or a sterile, dry mixture of Penicillin G Potassium with NLT 4.0% and NMT 5.0% of Sodium Citrate, of which NMT 0.15% may be replaced by Citric Acid. It has a potency of NLT 90.0% and NMT 120.0% of the labeled number of Penicillin G Units. In addition, where it contains Sodium Citrate it has a potency of NLT 1335 and NMT 1595 Penicillin G Units/mg.

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHY

Solution A: Acetone, 0.1 M citric acid, and 0.1 M sodium citrate (2:1:1)

Standard solution: Prepare a solution containing the equivalent of 12,000 Penicillin G Units/mL from USP Penicillin G Potassium RS in *Solution A*.

Sample solution: Nominally 12,000 Penicillin G Units/mL from Penicillin G Potassium for Injection in *Solution A*

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 μL

Developing solvent system: Toluene, dioxane, and glacial acetic acid (90:25:4)

Spray reagent 1: Starch TS

Spray reagent 2: Iodine TS diluted 1 in 10 with water

Analysis

Samples: *Standard solution* and *Sample solution*

Place the plate in a suitable chromatographic chamber. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow to air-dry. Spray the plate with *Spray reagent 1* followed by *Spray reagent 2*. Penicillin G appears as a white spot on a purple background.

Acceptance criteria: The *R_f* value of the penicillin G spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• PROCEDURE

Solution A: 0.01 M monobasic potassium phosphate

Mobile phase: Methanol and *Solution A* (40:60)

System suitability solution: 0.1 mg/mL each of USP Penicillin G Potassium RS and 2-phenylacetamide in water

Standard solution: 0.1 mg/mL of USP Penicillin G Potassium RS in water. This solution contains about 160 Penicillin G Units/mL. Shake as needed to dissolve.

Sample solution 1 (where it is represented as being in a single-dose container): Constitute Penicillin G Potassium for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a hypodermic needle and syringe, and dilute with water to obtain a solution containing nominally 160 Penicillin G Units/mL.

Sample solution 2 (where the label states the quantity of penicillin G in a given volume of constituted solution): Constitute Penicillin G Potassium for Injection as directed in the labeling. Dilute a suitable aliquot of the constituted solution with water to obtain a solution containing nominally 160 Penicillin G Units/mL.

Sample solution 3 (where it contains sodium citrate): Transfer about 50 mg of the Penicillin G Potassium for Injection to a 500-mL volumetric flask. Add 400 mL of water, and shake to dissolve. Dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 2-phenylacetamide and penicillin G are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between 2-phenylacetamide and penicillin G, *System suitability solution*

Column efficiency: NLT 1000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution 1*, *Sample solution 2*, or *Sample solution 3*

Perform the Assay on 10 containers where it is represented as being in a single-dose container and, if necessary, on 10 containers where the label states the quantity of penicillin G in a given volume of constituted solution. Use the individual results to determine the *Uniformity of Dosage Units* and the average of the results as the Assay value.

Calculate the percentage of the labeled number of Penicillin G Units in the container or in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response from *Sample solution 1* or *Sample solution 2*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of *Sample solution 1* or *Sample solution 2* (Penicillin G Units/mL)

P = potency of penicillin G in USP Penicillin G Potassium RS (Penicillin G Units/mg)

Calculate the potency, in Penicillin G Units/mg, of the Penicillin G Potassium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from *Sample solution 3*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin G Potassium for Injection in *Sample solution 3* (mg/mL)

P = potency of penicillin G in USP Penicillin G Potassium RS (Penicillin G Units/mg)

Acceptance criteria: 90.0%–120.0% of the labeled number of Penicillin G Units. Where Penicillin G Potassium for Injection contains sodium citrate it has a potency of NLT 1335 and NMT 1595 Penicillin G Units/mg.

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements. Perform the Assay on 10 containers where it is represented as being in a single-dose container and, if necessary, on 10 containers where the label states the quantity of penicillin G in a given volume of constituted solution. Use the individual results to determine the *Uniformity of Dosage Units* and the average of the results as the Assay value.

SPECIFIC TESTS

- **INJECTIONS AND IMPLANTED DRUG PRODUCTS (1), Specific Tests, Completeness and clarity of solutions:** At the time of use, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.01 USP Endotoxin Units/100 Penicillin G Units.
- **STERILITY TESTS (71):** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **CRYSTALLINITY (695):** Meets the requirements
- **PH (791)**

Sample solution: A solution containing 60 mg/mL. Where packaged for dispensing, use the solution constituted as directed in the labeling.

Acceptance criteria: 5.0–7.5. Where it is labeled as containing sodium citrate, the pH is between 6.0 and 8.5.

- **LOSS ON DRYING (731)**

Sample: 100 mg of Penicillin G Potassium for Injection
Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.

Acceptance criteria: NMT 1.5%

- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections

- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling (7)*, *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS**Change to read:**

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements (659)*, *Injection Packaging, Packaging for constitution* (CN 1-May-2017).
- **USP REFERENCE STANDARDS (11)**
USP Endotoxin RS
USP Penicillin G Potassium RS

Penicillin G Potassium for Oral Solution**DEFINITION**

Penicillin G Potassium for Oral Solution is a dry mixture of Penicillin G Potassium and one or more suitable buffers, colors, diluents, flavors, and preservatives. It contains NLT 90.0% and NMT 130.0% of the labeled number of Penicillin G Units when constituted as directed in the labeling.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

Solution A: Acetone, 0.1 M citric acid, and 0.1 M sodium citrate (2:1:1)

Standard solution: Prepare a solution containing the equivalent of 12,000 Penicillin G Units/mL from USP Penicillin G Potassium RS in *Solution A*

Sample solution: Shake a portion of it, containing nominally 100,000 Penicillin G Units, with 8 mL of *Solution A*

Chromatographic system

(See *Chromatography (621)*, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Toluene, dioxane, and glacial acetic acid (90:25:4)

Spray reagent 1: Starch TS

Spray reagent 2: Iodine TS diluted 1 in 10 with water

Analysis

Samples: *Standard solution* and *Sample solution*

Place the plate in a suitable chromatographic chamber. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow to air-dry. Spray the plate with *Spray reagent 1* followed by *Spray reagent 2*. Penicillin G appears as a white spot on a purple background.

Acceptance criteria: The R_f value of the penicillin G spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY**• PROCEDURE**

Standard solution: Prepare as directed in *Iodometric Assay—Antibiotics* (425), *Standard Preparation*, using USP Penicillin G Potassium RS.

Sample solution: Constitute Penicillin G Potassium for Oral Solution as directed in the labeling using *Buffer B.1* (see *Antibiotics—Microbial Assay* (81), *Media and Solutions, Solutions*). Dilute a suitable aliquot to obtain a solution containing nominally 2000 Penicillin G Units/mL.

Analysis

Samples: *Standard solution* and *Sample solution*

Pipet 2 mL of the *Sample solution* into each of two glass-stoppered, 125-mL conical flasks. Proceed as directed in *Iodometric Assay—Antibiotics* (425), *Procedure*, using one of the flasks to perform the *Blank Determination*.

Calculate the percentage of the labeled number of Penicillin G Units in the portion of Penicillin G Potassium for Oral Solution taken:

$$\text{Result} = (B - I) \times F \times [1/(D \times V)] \times 100$$

B = volume of 0.01 N sodium thiosulfate consumed in the *Blank Determination* (mL)

I = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation and Titration* (mL)

F = equivalency factor as calculated in the chapter (Penicillin G Unit/mL of 0.01 N sodium thiosulfate consumed by the *Standard solution*)

D = nominal concentration of penicillin G in the *Sample solution* (Penicillin G Units/mL)

V = volume of *Sample solution* used for the *Inactivation and Titration* (mL)

Acceptance criteria: 90.0%–130.0%

PERFORMANCE TESTS**• UNIFORMITY OF DOSAGE UNITS (905)**

For solids packaged in single-unit containers: Meets the requirements

• DELIVERABLE VOLUME (698): Meets the requirements**SPECIFIC TESTS****• pH (791)**

Sample solution: Constitute as directed in the labeling.
Acceptance criteria: 5.5–7.5

• WATER DETERMINATION (921), Method I: NMT 1.0%**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight containers.**• USP REFERENCE STANDARDS (11)**

USP Penicillin G Potassium RS

Penicillin G Potassium Tablets**DEFINITION**

Penicillin G Potassium Tablets contain NLT 90.0% and NMT 120.0% of the labeled number of Penicillin G Units.

IDENTIFICATION**• A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Acetone, 0.1 M citric acid, and 0.1 M sodium citrate (2:1:1)

Standard solution: 12,000 Penicillin G Units/mL from USP Penicillin G Potassium RS in *Diluent*

Sample solution: Nominally 12,500 Penicillin G Units/mL from Tablets in *Diluent*. Pass through a suitable filter.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Toluene, dioxane, and glacial acetic acid (90:25:4)

Spray reagent 1: Starch TS

Spray reagent 2: Iodine TS diluted 1 in 10 with water

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Sample solution* and the *Standard solution* to the plate, place in a suitable chromatographic chamber, and develop the chromatogram, using the *Developing solvent system*, until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow to air-dry. Spray the plate with *Spray reagent 1* followed by *Spray reagent 2*. Penicillin G appears as a white spot on a purple background.

Acceptance criteria: The *R_f* value of the penicillin G spot from the *Sample solution* corresponds to that from the *Standard solution*.

ASSAY**Change to read:****• PROCEDURE**

Standard solution: Prepare as directed in *Iodometric Assay—Antibiotics* (425), *Standard Preparation*, using USP Penicillin G Potassium RS.

Sample solution: Nominally 2000 Penicillin G Units/mL, prepared as follows. Place NLT 5 Tablets in a high-speed glass blender jar containing a measured volume of *Buffer B.1* (CN 1-May-2017), and blend for 4 ± 1 min.

Dilute a suitable aliquot with *Buffer B.1* (CN 1-May-2017).

Analysis: Proceed as directed in *Iodometric Assay—Antibiotics* (425), *Procedure*, using glass-stoppered, 125-mL conical flasks.

Calculate the percentage of the labeled number of Penicillin G Units in the portion of Tablets taken:

$$\text{Result} = (B - I) \times (F/2) \times (1/C_u) \times 100$$

B = volume of 0.01 N sodium thiosulfate consumed in *Blank Determination* (mL)

I = volume of 0.01 N sodium thiosulfate consumed in *Inactivation and Titration* of the *Sample solution* (mL)

F = factor as calculated in *Iodometric Assay—Antibiotics* (425), *Calculations*

C_u = nominal concentration of penicillin G in the *Sample solution* (Penicillin G Units/mL)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS**• DISSOLUTION (711)**

Medium: pH 6.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 75 rpm

Time: 60 min

Standard solution: 400 Penicillin G Units/mL from USP Penicillin G Potassium RS in *Medium*

Sample solution: Use a filtered portion of the solution under test.

Solution A: A 1-in-1000 solution of polyoxyethylene (23) lauryl ether in water

Solution B: Dissolve 20 g of hydroxylamine hydrochloride in 5 mL of *Solution A*, and add water to make 1000 mL.

Buffer: 26 mg/mL of sodium hydroxide and 3.1 mg/mL of sodium acetate in water

Ferric nitrate solution: Suspend 233 g of ferric nitrate in about 600 mL of water, add 2.8 mL of sulfuric acid, stir until the ferric nitrate is dissolved, add 1 mL of polyoxyethylene (23) lauryl ether, dilute with water to 1000 mL, and mix.

Apparatus: Automatic analyzer (Figure 1) consisting of (1) a liquid sampler, (2) a proportioning pump, (3) suitable spectrophotometers equipped with matched flow cells and analysis capability at 480 nm, (4) a means of recording spectrophotometric readings and/or a computer for data retrieval and calculation, and (5) a manifold consisting of the components illustrated in the figure.

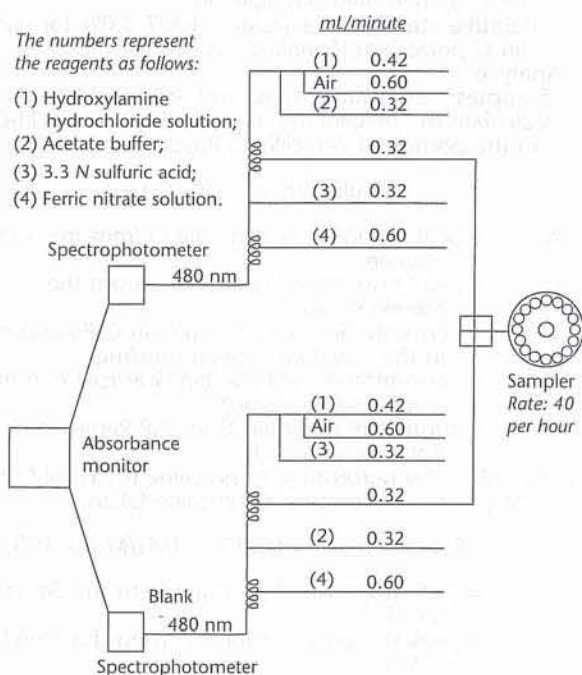


Figure 1

Analysis: With the sample line pumping water, the other lines pumping their respective reagents, and the spectrophotometer set at 480 nm, standardize the system until a steady absorbance baseline has been established. Transfer portions of the *Standard solution* and the *Sample solution* to sampler cups, and place in the sampler. Start the sampler, and conduct determinations of the *Standard solution* and the *Sample solution*, typically at the rate of 40 per h, using a ratio of about 2:1 for sample and wash time.

Calculate the percentage of the labeled amount of Penicillin G Units dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (Penicillin G Units/mL)

V = volume of *Medium*, 900 mL

L = label claim (Penicillin G Units/Tablet)

Tolerances: NLT 70% (Q) of the labeled amount of Penicillin G Units is dissolved.

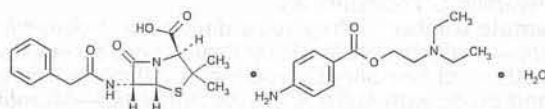
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Penicillin G Potassium RS

Penicillin G Procaine

Change to read:



$C_{16}H_{18}N_2O_4S \cdot C_{13}H_{20}N_2O_2 \cdot H_2O$ 588.72

$C_{16}H_{18}N_2O_4S \cdot C_{13}H_{20}N_2O_2$ 570.71

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino-], 2S-(2 α ,5 α ,6 β)-, Δ compound, Δ USP40 with 2-(diethylamino)ethyl 4-aminobenzoate (1:1) monohydrate;
(2S,5R,6R)-3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with 2-(diethylamino)ethyl *p*-aminobenzoate (1:1) monohydrate [6130-64-9].

Anhydrous [54-35-3].

DEFINITION

Penicillin G Procaine has a potency of NLT 900 Penicillin G Units/mg and NMT 1050 Penicillin G Units/mg.

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHY

Solution A: Acetone, 0.1 M citric acid, and 0.1 M sodium citrate (2:1:1)

Standard solution 1: Prepare a solution containing the equivalent of 12,000 Penicillin G Units/mL, from USP Penicillin G Potassium RS in *Solution A*.

Standard solution 2: 5 mg/mL of USP Procaine Hydrochloride RS in *Solution A*

Sample solution: Nominally 12,000 Penicillin G Units/mL from Penicillin G Procaine in *Solution A*

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 μ L

Developing solvent system: Toluene, dioxane, and glacial acetic acid (90:25:4)

Spray reagent 1: Starch TS

Spray reagent 2: Iodine TS diluted 1 in 10 with water

Spray reagent 3: 50 mg/mL of *p*-dimethylaminobenzaldehyde in methanol

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Proceed as directed in the chapter. Develop the chromatogram until the solvent has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow to air-dry. Examine the plate under short- and long-wavelength UV light, noting the positions of the spots. Spray the plate with *Spray reagent 1* followed by *Spray reagent 2*. Penicillin G appears as a white spot on a purple background. Spray the location of the spots visualized with UV light with *Spray reagent 3*. Procaine appears as a bright yellow spot.

Acceptance criteria: The R_f value of the penicillin G spot from the *Sample solution* corresponds to that from *Standard solution 1*. The R_f value of the procaine spot from the *Sample solution* corresponds to that from *Standard solution 2*.

ASSAY

Change to read:

• PROCEDURE

Standard solution: Prepare as directed in *Iodometric Assay—Antibiotics* (425), *Standard Preparation*, using USP Penicillin G Potassium RS.

Sample solution: Prepare as directed in *Iodometric Assay—Antibiotics* (425), *Assay Preparation*, except dissolve 100 mg of Penicillin G Procaine in 2.0 mL of methanol, and dilute with *Buffer B.1* (see *Antibiotics—Microbial Assays* (81))^{▲USP40} to obtain a solution containing 2000 Penicillin G Units/mL.

Analysis: Pipet 2 mL of the *Sample solution* into each of two glass-stoppered, 125-mL conical flasks. Use one of these to perform the *Blank Determination*. Proceed as directed in *Iodometric Assay—Antibiotics* (425), *Procedure*.

Calculate the potency, in Penicillin G Units/mg, of the Penicillin G Procaine taken:

$$\text{Result} = (B - I) \times F \times 1/(D \times V) \times 100$$

B = volume of 0.01 N sodium thiosulfate consumed in the *Blank Determination* (mL)

I = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation and Titration* (mL)

F = equivalency factor as calculated in *Procedure* in the chapter (Penicillin G Units/mL of 0.01 N sodium thiosulfate consumed by the *Standard solution*)

D = nominal concentration of Penicillin G in the *Sample solution* (Penicillin G Units/mL)

V = volume of the *Sample solution* used for the *Inactivation and Titration* (mL)

Acceptance criteria: 900–1050 Penicillin G Units/mg

SPECIFIC TESTS

Change to read:

• CONTENT OF PENICILLIN G AND PROCAINE

Solution A: [▲]Phosphoric acid diluted 1 in 10 with water^{▲USP40}

Mobile phase: Dissolve 14 g of monobasic potassium phosphate and 6.5 g of [▲]tetrabutylammonium hydroxide, 40% in water, [▲]USP40 in 700 mL of water. Adjust with 1 N potassium hydroxide to a pH of 7.0, and dilute with water to 1000 mL. Mix 500 mL of this solution, 250 mL of acetonitrile, and 250 mL of water. Adjust with 1 N potassium hydroxide or *Solution A* to a pH of 7.5 ± 0.05 , and pass through a suitable filter.

Standard solution: 0.8 mg/mL of USP Penicillin G Potassium RS and 0.54 mg/mL of USP Procaine Hydrochloride RS in *Mobile phase*

System suitability solution: 2.4 mg/mL of [▲]USP Penicillin V Potassium RS^{▲USP40} in *Mobile phase*. Mix the resultant solution with *Standard solution* (1:3).

Sample solution: Transfer 70 mg of Penicillin G Procaine to a 50-mL volumetric flask. Add 30 mL of *Mobile phase*, sonicate to dissolve, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: [▲]3.9-mm^{▲USP40} × 30-cm; 10-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—[▲]The relative retention times for procaine, penicillin G, and penicillin V are about 0.4, 1.0, and 1.5, respectively.^{▲USP40}]

Suitability requirements

Resolution: NLT 2.0 between penicillin G and penicillin V, *System suitability solution*

Relative standard deviation: NMT 3.0% for penicillin G potassium, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of penicillin G (C₁₆H₁₈N₂O₄S) in the portion of Penicillin G Procaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times G_S$$

r_U = peak response of penicillin G from the *Sample solution*

r_S = peak response of penicillin G from the *Standard solution*

C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin G Procaine in the *Sample solution* (mg)

G_S = content of penicillin G in USP Penicillin G Potassium RS (%)

Calculate the percentage of procaine (C₁₃H₂₀N₂O₂) in the portion of Penicillin G Procaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of procaine from the *Sample solution*

r_S = peak response of procaine from the *Standard solution*

C_S = concentration of USP Procaine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin G Procaine in the *Sample solution* (mg)

M_{r1} = molecular weight of procaine, 236.32

M_{r2} = molecular weight of procaine hydrochloride, 272.78

Acceptance criteria: See *Table 1*.

Table 1

Penicillin G	51.0%–59.6%
Procaine	37.5%–43.0%

- **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Penicillin G Procaine is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.01 USP Endotoxin Unit/100 Penicillin G Units.

Change to read:

- **STERILITY TESTS (71):** Where the label states that Penicillin G Procaine is sterile, it meets the requirements. [▲]If the test for *Membrane Filtration* is used, perform the procedure as directed in the chapter with the following exceptions. Use *Fluid A* to which has been added sufficient sterile penicillinase to inactivate the penicillin G, and swirl the vessel until solution is complete before filtering.^{▲USP40}

- **CRYSTALLINITY** (695): Meets the requirements

Change to read:

- **pH** (791)
Sample solution: A saturated ^{▲USP40} solution containing about 300 mg/mL of Penicillin G Procaine in water
Acceptance criteria: 5.0–7.5
- **WATER DETERMINATION** (921), Method I: 2.8%–4.2%

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** [▲]Where it is intended for use in preparing injectable dosage forms, preserve as directed in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution*. ^{▲USP40}
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

Change to read:

- **USP REFERENCE STANDARDS** (11)
USP Endotoxin RS
USP Penicillin G Potassium RS
[▲]USP Penicillin V Potassium RS ^{▲USP40}
USP Procaine Hydrochloride RS

Penicillin G Procaine Intramammary Infusion

» Penicillin G Procaine Intramammary Infusion is a suspension of Penicillin G Procaine in a suitable vegetable oil vehicle. It may contain one or more buffers, dispersants, preservatives, and thickening agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of penicillin G.

Packaging and storage—Preserve in well-closed disposable syringes.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—
USP Penicillin G Potassium RS
USP Penicillin G Procaine RS

Identification—Transfer a portion of it, equivalent to about 100,000 Penicillin G Units, to a test tube, add 25 mL of methanol, and shake. Allow to separate, and use the methanol layer as the test solution. Prepare a Standard solution of USP Penicillin G Procaine RS in methanol containing about 4.5 mg per mL. Apply separately 10 μ L of each solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of butanol, isopropyl alcohol, acetone, and water (4:4:2:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Expose the plate to iodine vapors in a closed chamber for about 15 minutes, and locate the spots: the R_f values and colors of the two principal spots

obtained from the test solution correspond to those obtained from the Standard solution.

Water Determination, Method I (921): not more than 1.4%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Change to read:

Assay—Proceed as directed under *Antibiotics—Microbial Assays* (81), expelling the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of [•]Buffer B.1 [•](CN 1-May-2017) and 1.0 mL of polysorbate 80, and blending for 3 to 5 minutes. Allow to stand for about 10 minutes, and dilute an accurately measured volume of the aqueous phase quantitatively and stepwise with [•]Buffer B.1 [•](CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Penicillin G Procaine Injectable Suspension

» Penicillin G Procaine Injectable Suspension is a sterile suspension of Penicillin G, Procaine or, where labeled for veterinary use only of sterile penicillin G procaine, in Water for Injection and contains one or more suitable buffers dispersants, or suspending agents, and a suitable preservative. It may contain procaine hydrochloride in a concentration not exceeding 2.0 percent, and may contain one or more suitable stabilizers. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of penicillin G, the labeled amount being not less than 300,000 Penicillin G Units per mL or per container.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass, in a refrigerator.

Labeling—Where it is intended for veterinary use only, the label so states.

USP Reference standards (11)—
USP Endotoxin RS
USP Penicillin G Potassium RS
USP Procaine Hydrochloride RS

Identification—It responds to the *Identification* test under *Penicillin G Procaine*.

Crystallinity (695) (where it is prepared from penicillin G procaine and is labeled for veterinary use only): meets the requirements, the dried residue prepared as directed in the test for *Penicillin G and procaine contents* being used.

Bacterial Endotoxins Test (85)—It contains not more than 0.01 USP Endotoxin Unit per 100 Penicillin G Units.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to use a portion of specimen from each container equivalent to 300,000 Penicillin G Units, instead of the minimum volume specified in the *Table 2, Minimum Quantity to be Used for Each Medium*, and to use *Fluid A* to which has been added sufficient sterile penicillinase to inactivate the penicillin G and to swirl the vessel until solution is complete before filtering. If the Injectable Suspension contains lecithin, use *Fluid D*. If it contains carboxymethylcellulose sodium, add sufficient sterile carboxymethylcellulase to *Fluid A* or *Fluid D* to dissolve the

carboxymethylcellulose sodium before filtering. If it does not dissolve completely, proceed as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, except to use Fluid Thioglycollate Medium and Soybean-Casein Digest Medium containing an amount of sterile penicillinase sufficient to inactivate the penicillin G in each vessel.

pH (791): between 5.0 and 7.5.

Penicillin G and procaine contents (where it is prepared from penicillin G procaine and is labeled for veterinary use only)—Dilute a portion of it, equivalent to about 300,000 Penicillin G Units, with water to obtain a volume of 10 mL, centrifuge, and remove and discard the supernatant. Resuspend the sediment in 10 mL of water, centrifuge, and remove and discard the supernatant. Dry the sediment in a vacuum desiccator containing silica gel for 18 hours at a temperature not exceeding 25°. The dried material meets the requirements of the test for *Penicillin G and procaine contents* under *Penicillin G Procaine*. [NOTE—Reserve a portion of the dried material for the test for *Crystallinity*.]

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Change to read:

Assay—

Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* (425).

Assay preparation 1 (where it is represented as being in a single-dose container)—Withdraw all of the withdrawable contents of the Injectable Suspension, using a suitable hypodermic needle and syringe, and dilute quantitatively with **Buffer B.1** (CN 1-May-2017) to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Assay preparation 2 (where the label states the quantity of penicillin G procaine in a given volume of Injectable Suspension)—Dilute an accurately measured volume of Injectable Suspension quantitatively with **Buffer B.1** (CN 1-May-2017) to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2.0 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425). Calculate the quantity, in Penicillin G Units, in the container, or in the portion of Injectable Suspension taken, by the formula:

$$(L / 2D)(F)(B - I)$$

in which *L* is the labeled quantity in Penicillin G Units, in the container, or in the volume of Injectable Suspension taken; and *D* is the concentration, in Penicillin G Units per mL, of *Assay preparation 1*, or of *Assay preparation 2*, on the basis of the labeled quantity in the container, or in the portion of Injectable Suspension taken, respectively, and the extent of dilution.

Penicillin G Procaine for Injectable Suspension

» Penicillin G Procaine for Injectable Suspension is a sterile mixture of Penicillin G Procaine and

one or more suitable buffers, dispersants, or suspending agents, and preservatives. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of penicillin G, the labeled amount being not less than 300,000 Penicillin G Units per container or per mL of constituted Suspension.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass.

USP Reference standards (11)—

USP Endotoxin RS

USP Penicillin G Potassium RS

USP Procaine Hydrochloride RS

Identification—It responds to the *Identification* test under *Penicillin G Procaine*.

pH (791): between 5.0 and 7.5, when constituted as directed in the labeling.

Water Determination, Method I (921): between 2.8% and 4.2%.

Other requirements—It meets the requirements for *Bacterial endotoxins* and *Sterility* under *Penicillin G Procaine Injectable Suspension*. It meets also the requirements under *Injections and Implanted Drug Products* (1) and *Uniformity of Dosage Units* (905).

Change to read:

Assay—

Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* (425).

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Penicillin G Procaine for Injectable Suspension as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with **Buffer B.1** (CN 1-May-2017) to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Assay preparation 2 (where the label states the quantity of penicillin G procaine in a given volume of constituted suspension)—Constitute Penicillin G Procaine for Injectable Suspension as directed in the labeling. Dilute an accurately measured volume of the constituted injectable suspension quantitatively with **Buffer B.1** (CN 1-May-2017) to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425). Calculate the quantity, in Penicillin G Units, in the container, or in the portion of constituted injectable suspension taken by the formula:

$$(L / 2D)(F)(B - I)$$

in which *L* is the labeled quantity, in Penicillin G Units, in the container, or in the volume of constituted injectable suspension taken, and *D* is the concentration, in Penicillin G Units per mL, of *Assay preparation 1* or of *Assay preparation 2* on the basis of the labeled quantity in the container or in the portion of constituted injectable suspension taken, respectively, and the extent of dilution.

Penicillin G Procaine and Dihydrostreptomycin Sulfate Intramammary Infusion

» Penicillin G Procaine and Dihydrostreptomycin Sulfate Intramammary Infusion is a suspension of Penicillin G Procaine and Dihydrostreptomycin Sulfate in a suitable vegetable oil vehicle. It may contain suitable gelling and thickening agents. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amounts of Penicillin G Units and of dihydrostreptomycin ($C_{21}H_{41}N_7O_{12}$).

Packaging and storage—Preserve in well-closed, disposable syringes.

Labeling—Label it to indicate that it is intended for veterinary use only.

USP Reference standards (11)—
USP Dihydrostreptomycin Sulfate RS
USP Penicillin G Potassium RS
USP Penicillin G Procaine RS

Identification—

A: It responds to the *Identification* test under *Penicillin G Procaine Intramammary Infusion*.

B: Place a portion of it, equivalent to about 100 mg of dihydrostreptomycin, in a separator, add 20 mL of chloroform and 20 mL of water, and shake by mechanical means for 15 minutes. Allow to separate, and discard the lower chloroform layer. Repeat the extraction with a 20-mL portion of chloroform, discarding the chloroform layer. Use the aqueous layer as the test solution. Prepare a Standard solution of USP Dihydrostreptomycin Sulfate RS in water containing 6.5 mg per mL. Apply separately 30 μ L of each solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-propyl alcohol, water, pyridine, and glacial acetic acid (15:12:10:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a reagent prepared by dissolving 2 g of ninhydrin in 100 mL of alcohol and adding 20 mL of glacial acetic acid, heat the plate at 110° for 10 minutes, and examine the chromatograms: the R_f value and color of the principal spot obtained from the test solution correspond to those obtained from the Standard solution.

Water Determination, *Method I* (921): not more than 1.4%, 20 mL of a mixture of toluene and methanol (7:3) being used in the titration vessel in place of methanol.

Change to read:

Assay for penicillin G—Proceed as directed for penicillin G under *Antibiotics—Microbial Assays* (81), expelling the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of *Buffer B.1* (CN 1-May-2017) and 1.0 mL of polysorbate 80, and blending for 3 to 5 minutes. Allow to stand for about 10 minutes, and dilute an accurately measured volume of the aqueous phase quantitatively and stepwise with *Buffer B.1* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of penicillin G assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for dihydrostreptomycin—Proceed as directed for the cylinder-plate assay for dihydrostreptomycin under *Antibiotics—Microbial Assays* (81), expelling the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of *Buffer B.3* (CN 1-May-2017) and 1.0 mL of polysorbate 80, and blending for 3 to 5 minutes. Allow to stand for about 10 minutes, and to an accurately measured volume of the aqueous phase add an accurately measured volume of penicillinase sufficient to inactivate the penicillin G contained therein. Dilute this solution quantitatively with *Buffer B.3* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of dihydrostreptomycin assumed to be equal to the median dose level of the Standard, and store at 37° for 30 minutes before filling the cylinders.

Penicillin G Procaine and Dihydrostreptomycin Sulfate Injectable Suspension

» Penicillin G Procaine and Dihydrostreptomycin Sulfate Injectable Suspension is a sterile suspension of Penicillin G Procaine in a solution of Dihydrostreptomycin Sulfate in Water for Injection, and contains one or more suitable buffers, preservatives, and dispersing or suspending agents. It may contain Procaine Hydrochloride in a concentration not exceeding 2.0 percent, and it may contain one or more suitable stabilizers. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of Penicillin G Units and of dihydrostreptomycin ($C_{21}H_{41}N_7O_{12}$).

Packaging and storage—Preserve in single-dose or multiple-dose, tight containers.

Labeling—Label it to indicate that it is intended for veterinary use only.

USP Reference standards (11)—
USP Dihydrostreptomycin Sulfate RS
USP Endotoxin RS
USP Penicillin G Potassium RS

Identification—It responds to *Identification* tests A and B under *Penicillin G Procaine, Dihydrostreptomycin Sulfate, Chlorpheniramine Maleate, and Dexamethasone Suspension*.

Bacterial Endotoxins Test (85)—It contains not more than 0.01 USP Endotoxin Unit per 100 Penicillin G Units.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to use a portion of specimen from each container equivalent to 300,000 Penicillin G Units, instead of the minimum volume specified in the *Table 2, Minimum Quantity to be Used for Each Medium* and to use *Fluid A* to which has been added sufficient sterile penicillinase to inactivate the penicillin G and to swirl the vessel until solution is complete before filtering. If the Injectable Suspension contains lecithin, use *Fluid D* to which has been added sufficient penicillinase to inactivate the penicillin G and to swirl the vessel until solution is complete before filtering. If it contains carboxymethylcellulose sodium, add also sufficient sterile carboxymethylcellulase to *Fluid A* or *Fluid D* to dissolve the carboxymethylcellulose sodium before filtering. If it does not dissolve completely, proceed as directed for *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, except to use *Fluid*

Thioglycollate Medium containing an amount of sterile penicillinase sufficient to inactivate the penicillin G in each vessel.

pH (791): between 5.0 and 8.0.

Change to read:

Assay for penicillin G—

Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* (425).

Assay preparation—Dilute an accurately measured volume of Injectable Suspension quantitatively with **Buffer B.1** (CN 1, May-2017) to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425), except in the *Blank Determination* to add 0.1 mL of 1.2 N hydrochloric acid immediately before the 10.0 mL of 0.01 N iodine VS. Calculate the quantity, in Penicillin G Units, in the portion of Injectable Suspension taken by the formula:

$$(L / 2D)(F)(B - I)$$

in which *L* is the labeled quantity, in Penicillin G Units, in the volume of Injectable Suspension taken, and *D* is the concentration, in Penicillin G Units per mL, of the *Assay preparation*, on the basis of the labeled quantity in the portion of Injectable Suspension taken and the extent of dilution, and the other terms are as defined therein.

Assay for dihydrostreptomycin—Proceed as directed for the turbidimetric assay for dihydrostreptomycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Injectable Suspension diluted quantitatively with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Penicillin G Procaine, Dihydrostreptomycin Sulfate, Chlorpheniramine Maleate, and Dexamethasone Injectable Suspension

» Penicillin G Procaine, Dihydrostreptomycin Sulfate, Chlorpheniramine Maleate, and Dexamethasone Injectable Suspension is a sterile suspension of Penicillin G Procaine and Dexamethasone in a solution of Sterile Dihydrostreptomycin Sulfate and Chlorpheniramine Maleate in Water for Injection. It contains one or more suitable buffers, preservatives, and dispersing or suspending agents. It may contain Procaine Hydrochloride in a concentration not exceeding 2.0 percent, and it may contain one or more suitable stabilizers. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of Penicillin G Units and of dihydrostreptomycin ($C_{21}H_{41}N_7O_{12}$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) and of dexamethasone ($C_{22}H_{29}FO_5$).

Packaging and storage—Preserve in single-dose or multiple-dose, tight containers, in a cool place.

Labeling—Label it to indicate that it is intended for veterinary use only.

USP Reference standards (11)—

USP Chlorpheniramine Maleate RS
USP Dexamethasone RS
USP Dihydrostreptomycin Sulfate RS
USP Endotoxin RS
USP Penicillin G Potassium RS
USP Penicillin G Procaine RS

Identification—

A: Transfer, with the aid of water, a portion of the Injectable Suspension, freshly mixed and free from air bubbles, equivalent to about 400,000 Penicillin G Units, to a separator, add 50 mL of chloroform, and shake by mechanical means for 15 minutes. Allow to separate, and filter the lower chloroform layer through about 4 g of anhydrous sodium sulfate supported on a pledget of glass wool, collecting the filtrate in a 100-mL volumetric flask. Repeat the extraction with two 25-mL portions of chloroform, combining the filtrates in the 100-mL volumetric flask. Dilute with chloroform to volume, and mix. [NOTE—Retain the aqueous phase for *Identification test B.*] Prepare a Standard solution of USP Penicillin G Procaine RS in chloroform containing about 4.5 mg per mL. Apply separately 10 μ L of each solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of butanol, isopropyl alcohol, acetone, and water (4:4:2:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Expose the plate to iodine vapors in a closed chamber for about 15 minutes, and locate the spots: the *R_F* values and colors of the two principal spots obtained from the test solution correspond to those obtained from the Standard solution.

B: Dilute the aqueous phase retained from *Identification test A* with water to obtain a test solution containing about 5 mg of dihydrostreptomycin per mL. Prepare a Standard solution of USP Dihydrostreptomycin Sulfate RS in water containing 6.5 mg per mL. Apply separately 30 μ L of each solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-propyl alcohol, water, pyridine, and glacial acetic acid (15:12:10:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a reagent prepared by dissolving 2 g of ninhydrin in 100 mL of alcohol and adding 20 mL of glacial acetic acid, heat the plate at 110° for 10 minutes, and examine the chromatograms: the *R_F* value and color of the principal spot obtained from the test solution correspond to those obtained from the Standard solution.

C: The chromatogram of the *Assay preparation* obtained as directed in the *Assay for chlorpheniramine maleate* exhibits a major peak for chlorpheniramine, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* similarly determined, both relative to the internal standard.

D: The chromatogram of the *Assay preparation* obtained as directed in the *Assay for dexamethasone* exhibits a major peak for dexamethasone, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* similarly determined, both relative to the internal standard.

Bacterial Endotoxins Test (85)—It contains not more than 0.01 Endotoxin Unit per 100 Penicillin G Units.

pH (791): between 5.0 and 6.0.

Other requirements—It meets the requirements of the test for *Sterility* under *Penicillin G Procaine and Dihydrostreptomycin Sulfate Injectable Suspension*, and the requirements under *Injections and Implanted Drug Products* (1).

Change to read:

Assay for penicillin G—

Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* (425).

Assay preparation—Dilute an accurately measured volume of Injectable Suspension, freshly mixed and free from air bubbles, quantitatively with **Buffer B.1** (CN 1-May-2017) to yield a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425), except in the *Blank Determination* to add 0.1 mL of 1.2 N hydrochloric acid immediately before the 10.0 mL of 0.01 N iodine VS. Calculate the quantity, in Penicillin G Units, in the portion of Injectable Suspension taken by the formula:

$$(L / 2D)(F(B - I))$$

in which *L* is the labeled quantity, in Penicillin G Units, in the volume of Injectable Suspension taken, and *D* is the concentration, in Penicillin G Units per mL, of the *Assay preparation*, on the basis of the labeled quantity in the portion of Injectable Suspension taken and the extent of dilution, and the other terms are as defined therein.

Assay for dihydrostreptomycin—Proceed as directed for the turbidimetric assay for dihydrostreptomycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Injectable Suspension, freshly mixed and free from air bubbles, diluted quantitatively with water to yield a *Test Dilution* having a concentration of dihydrostreptomycin assumed to be equal to the median dose level of the *Standard*.

Assay for chlorpheniramine maleate—

Internal standard solution—Prepare a solution of brompheniramine maleate in water having a concentration of about 7 mg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a stock solution having a known concentration of about 6 mg per mL. Transfer 5.0 mL of this solution to a 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution*, and adjust with sodium hydroxide solution (1 in 20) to a pH of about 10. Add 25.0 mL of hexanes, place the cap on the tube, shake for about 2 minutes, and centrifuge. Use the upper hexanes layer as the *Standard preparation*.

Assay preparation—Transfer an accurately measured volume of Injectable Suspension, freshly mixed and free from air bubbles, equivalent to about 30 mg of chlorpheniramine maleate, to a 50-mL centrifuge tube. Proceed as directed under *Standard preparation*, beginning with "Add 5.0 mL of *Internal standard solution*." Use the upper hexanes layer as the *Assay preparation*.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector, and contains a 4-mm × 1.8-m glass column packed with 1.2% liquid phase G16 and 0.5% potassium hydroxide on 100- to 120-mesh support S1A. The column is maintained isothermally at about 180°, and the injection port and the detector block are maintained at about 200°. Dry nitrogen is used as the carrier gas at a flow rate of about 50 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Proce-*

cedure: the resolution, *R*, between the analyte and internal standard peaks is not less than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 1.5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.75 for chlorpheniramine and 1.0 for brompheniramine. Calculate the quantity, in mg, of chlorpheniramine maleate (C₁₆H₁₉ClN₂ · C₄H₄O₄) in each mL of the Injectable Suspension taken by the formula:

$$5(C / V)(R_u / R_s)$$

in which *C* is the concentration of USP Chlorpheniramine Maleate RS in the stock solution used to prepare the *Standard preparation*, *V* is the volume, in mL, of Injectable Suspension taken, and *R_u* and *R_s* are the peak response ratios of the chlorpheniramine maleate peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for dexamethasone—

Mobile phase—Prepare a suitable filtered mixture of water and acetonitrile (2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard solution—Dissolve about 30 mg of beclomethasone in 2 mL of methanol in a 50-mL volumetric flask, dilute with methylene chloride to volume, and mix.

Standard preparation—Transfer about 25 mg of USP Dexamethasone RS, accurately weighed, to a 50-mL volumetric flask. Add about 1 mL of methanol, swirl to dissolve, dilute with methylene chloride to volume, and mix. Transfer 5.0 mL of this solution to a suitable flask, and add 5.0 mL of *Internal standard solution*. Heat the flask on a steam bath, and evaporate under a stream of nitrogen just to dryness. Add 10.0 mL of methanol to the flask, and swirl to dissolve the residue. This *Standard preparation* contains about 0.25 mg of USP Dexamethasone RS and 0.3 mg of beclomethasone per mL.

Assay preparation—Transfer an accurately measured volume of Injectable Suspension, freshly mixed and free from air bubbles, equivalent to about 2.5 mg of dexamethasone, to a separator containing 50 mL of 0.1 N hydrochloric acid, add 5.0 mL of *Internal standard solution*, and extract with four 25-mL portions of methylene chloride, combining the extracts in a second separator. Wash the combined extracts with 50 mL of sodium bicarbonate solution (1 in 20), filtering the lower methylene chloride layer through about 4 g of anhydrous sodium sulfate supported on a cotton pledget previously washed with methylene chloride, and collecting the filtrate in a suitable flask. Wash the aqueous layer with 25 mL of methylene chloride, and filter the lower methylene chloride layer through the same filter, collecting the filtrate in the same flask. Heat the flask on a steam bath, and evaporate under a stream of nitrogen just to dryness. Add 10.0 mL of methanol, and swirl to dissolve the residue.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, of the analyte and the internal standard peaks is not less than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.8 for dexamethasone and 1.0 for beclomethasone. Calculate the quantity, in mg, of dex-

amethasone ($C_{22}H_{29}FO_5$) in each mL of the Injectable Suspension taken by the formula:

$$10(C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*, V is the volume, in mL, of Injectable Suspension taken, and R_U and R_S are the peak response ratios of the dexamethasone peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Penicillin G Procaine, Dihydrostreptomycin Sulfate, and Prednisolone Injectable Suspension

» Penicillin G Procaine, Dihydrostreptomycin Sulfate, and Prednisolone Injectable Suspension is a sterile suspension of Penicillin G Procaine and Prednisolone in a solution of Dihydrostreptomycin Sulfate in Water for Injection. It contains one or more suitable buffers, dispersants, preservatives, and suspending agents. It may contain not more than 2.0 percent of procaine hydrochloride, and one or more suitable stabilizing agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled number of Penicillin G Units, not less than 90.0 percent and not more than 115.0 percent of the labeled amount of dihydrostreptomycin ($C_{21}H_{41}N_7O_{12}$), and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of prednisolone ($C_{21}H_{28}O_5$).

Packaging and storage—Preserve in single-dose or multiple-dose, tight containers.

Labeling—Label it to indicate that it is intended for veterinary use only, and is not to be used in animals to be slaughtered for human consumption.

USP Reference standards (11)—
USP Dihydrostreptomycin Sulfate RS
USP Endotoxin RS
USP Penicillin G Potassium RS
USP Prednisolone RS

Identification—It responds to *Identification tests A and B* under *Penicillin G Procaine, Dihydrostreptomycin Sulfate, Chlorpheniramine Maleate, and Dexamethasone Injectable Suspension*.

Bacterial Endotoxins Test (85)—It contains not more than 0.01 Endotoxin Unit per 100 Penicillin G Units.

Other requirements—It meets the requirements of the test for *Sterility*, and for *pH* under *Penicillin G Procaine and Dihydrostreptomycin Sulfate Injectable Suspension*.

Change to read:

Assay for penicillin G—

Standard preparation—Using USP Penicillin G Potassium RS, prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* (425).

Assay preparation—Dilute an accurately measured volume of Injectable Suspension quantitatively with *Buffer B.1* (CN.1. May-2017) to obtain a solution containing about 2000 Penicillin G Units per mL. Pipet 2 mL of this solution into each of two glass-stoppered, 125-mL conical flasks.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425), except in the *Blank Determination* to add 0.1 mL of 1.2 N hydrochloric acid immediately before the 10.0 mL of 0.01 N iodine VS. Calculate the quantity, in Penicillin G Units, in the portion of Injectable Suspension taken by the formula:

$$(L/2D)(F(B-I))$$

in which L is the labeled quantity, in Penicillin G Units, in the volume of Injectable Suspension taken, D is the concentration, in Penicillin G Units per mL, of the *Assay preparation*, on the basis of the labeled quantity in the portion of Injectable Suspension taken and the extent of dilution, and the other terms are as defined therein.

Assay for dihydrostreptomycin—Proceed as directed for the turbidimetric assay for dihydrostreptomycin as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Injectable Suspension diluted quantitatively with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for prednisolone—

Standard preparation—Prepare as directed for *Standard Preparation* under *Single-steroid Assay* (511), using USP Prednisolone RS.

Assay preparation—Transfer to a separator an accurately measured volume of Injectable Suspension, and add 15 mL of water. Extract with three 25-mL portions and finally with one 20-mL portion of chloroform, filtering each portion through chloroform-washed cotton into a 100-mL volumetric flask. Add chloroform to volume, and mix. Pipet 20 mL of this solution into a suitable glass-stoppered flask or tube, evaporate the chloroform on a steam bath just to dryness, cool, and dissolve the residue in 2.0 mL, accurately measured, of a mixture of equal volumes of chloroform and alcohol.

Procedure—Proceed as directed for *Single-Steroid Assay* (511), using *Solvent A* to develop the chromatogram. Calculate the quantity, in mg, of prednisolone ($C_{21}H_{28}O_5$) in each mL of the Injectable Suspension taken by the formula:

$$0.01(C/V)(A_U/A_S)$$

in which the terms are as defined therein.

Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension

» Penicillin G Procaine, Neomycin and Polymyxin B Sulfates, and Hydrocortisone Acetate Topical Suspension is a suspension of Penicillin G Procaine, Neomycin Sulfate, Polymyxin B Sulfate and Hydrocortisone Acetate in Peanut Oil or Sesame Oil. It may contain one or more suitable dispersing and suspending agents. It contains not less than 90.0 percent and not more than 140.0 percent of the labeled amounts of Penicillin G Units, of neomycin, and of polymyxin B Units, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in well-closed containers.

Labeling—Label it to indicate that it is intended for veterinary use only.

USP Reference standards (11)—

USP Hydrocortisone Acetate RS
USP Neomycin Sulfate RS
USP Penicillin G Potassium RS
USP Polymyxin B Sulfate RS

Water Determination, Method I (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

Change to read:

Assay for penicillin G—Proceed as directed for penicillin G under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Topical Suspension blended for 2 minutes in a high-speed glass blender jar with 499.0 mL of *Buffer B.1* (CN 1-May-2017) and 1.0 mL of polysorbate 80. Allow to stand for 10 minutes, and dilute an accurately measured volume of the aqueous phase quantitatively and stepwise with *Buffer B.1* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of penicillin G assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Topical Suspension shaken in a separator with about 50 mL of ether, and extracted with four 20-mL portions of *Buffer B.3* (CN 1-May-2017). Combine the aqueous extracts, and dilute with *Buffer B.3* (CN 1-May-2017) to an appropriate volume to obtain a stock solution. To an accurately measured volume of this stock solution add an accurately measured volume of penicillinase sufficient to inactivate the penicillin G therein, heat at 37° for 30 minutes, and dilute quantitatively and stepwise with *Buffer B.3* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of neomycin assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Topical Suspension blended for 2 minutes in a high-speed glass blender jar containing 499.0 mL of *Buffer B.6* (CN 1-May-2017) and 1.0 mL of polysorbate 80. Allow to stand for 10 minutes, and to an accurately measured volume of the aqueous phase add an accurately measured volume of penicillinase sufficient to inactivate the penicillin G therein. Heat the solution at 37° for 30 minutes, and dilute quantitatively and stepwise with *Buffer B.6* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of polymyxin assumed to be equal to the median dose level of the Standard. Add to each test dilution of the Standard a quantity of USP Neomycin Sulfate RS dissolved in *Buffer B.6* (CN 1-May-2017) to obtain the same concentration of neomycin present in the *Test Dilution*.

Assay for hydrocortisone acetate—Using an accurately measured volume of Topical Suspension, proceed as directed in the Assay under *Hydrocortisone Acetate Lotion*.

Penicillin G Procaine and Novobiocin Sodium Intramammary Infusion

» Penicillin G Procaine and Novobiocin Sodium Intramammary Infusion is a suspension of Penicillin G Procaine and Novobiocin Sodium in a suitable vegetable oil vehicle. It contains a suitable preservative and suspending agent. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amounts of Penicillin G Units and novobiocin ($C_{31}H_{36}N_2O_{11}$).

Packaging and storage—Preserve in disposable syringes that are well-closed containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Novobiocin RS
USP Penicillin G Potassium RS

Water Determination, Method I (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

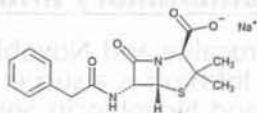
Change to read:

Assay for penicillin G—Proceed as directed for penicillin G under *Antibiotics—Microbial Assays* (81), except to use *Staphylococcus aureus* ATCC No. 12692 as the test organism. Prepare the inoculum by growing the organism at 32° to 35° for 24 hours on Medium 1 to which has been added a solution of novobiocin sodium, containing the equivalent of 2.5 mg of novobiocin per mL that has been filtered through a membrane filter having a 0.2-μm porosity, so that the medium contains the equivalent of 10 μg of novobiocin per mL. Use an inoculum composition of about 5 mL of stock suspension in each 100 mL of Medium 1. Expel the contents of a syringe of Intramammary Infusion into a high-speed glass blender jar containing 1.0 mL of polysorbate 80 and 499.0 mL of *Buffer B.1* (CN 1-May-2017), and blend for 3 to 5 minutes. Allow to stand for 10 minutes, and dilute an accurately measured volume of the aqueous phase quantitatively and stepwise with *Buffer B.1* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of penicillin G assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for novobiocin—Proceed as directed for novobiocin under *Antibiotics—Microbial Assays* (81), expelling the contents of a syringe of Intramammary Infusion into a high-speed blender jar containing 1.0 mL of polysorbate 80 and 499.0 mL of *Buffer B.3* (CN 1-May-2017), and blend for 3 to 5 minutes. Allow to stand for 10 minutes. To an accurately measured volume of the aqueous phase add sufficient penicillinase to inactivate the penicillin G therein, and dilute quantitatively and stepwise with *Buffer B.6* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of novobiocin assumed to be equal to the median dose level of the Standard. [NOTE—Store this *Test Dilution* at 37° for 30 minutes and allow to cool before using it to fill the cylinders on the plates.]

Penicillin G Sodium



$C_{16}H_{17}N_2NaO_4S$ 356.7
 4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-, [2S-(2 α ,5 α ,6 β)], monosodium salt;
 Monosodium (2S,5R,6R)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [69-57-8].

DEFINITION

Penicillin G Sodium has a potency of NLT 1500 and NMT 1750 Penicillin G Units/mg.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL** (191), *Sodium*: Meets the requirements

ASSAY

• PROCEDURE

Solution A: 0.01 M monobasic potassium phosphate

Mobile phase: Methanol and *Solution A* (40:60)

System suitability solution: 0.1 mg/mL each of USP Penicillin G Potassium RS and 2-phenylacetamide in water

Standard solution: 0.1 mg/mL of USP Penicillin G Potassium RS in water. Shake as needed to dissolve. This solution contains about 160 Penicillin G Units/mL.

Sample solution: 0.1 mg/mL of Penicillin G Sodium in water

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 10-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 2-phenylacetamide and penicillin G are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between 2-phenylacetamide and penicillin G, *System suitability solution*

Column efficiency: NLT 1000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the potency of penicillin G sodium, in Penicillin G Units/mg, in the portion of Penicillin G Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response of penicillin G from the *Sample solution*

r_S = peak response of penicillin G from the *Standard solution*

C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin G Sodium in the *Sample solution* (mg/mL)

P = potency of penicillin G in USP Penicillin G Potassium RS (Penicillin G Units/mg)

Acceptance criteria: 1500–1750 Penicillin G Units/mg

SPECIFIC TESTS

- **CRYSTALLINITY** (695): Meets the requirements
- **PH** (791)
Sample solution: 60 mg/mL of Penicillin G Sodium in water
Acceptance criteria: 5.0–7.5
- **LOSS ON DRYING** (731)
Sample: 100 mg of Penicillin G Sodium
Analysis: Dry the *Sample* in a capillary-stoppered bottle under a vacuum at a pressure NMT 5 mm of mercury at 60° for 3 h.
Acceptance criteria: NMT 1.5%
- **STERILITY TESTS** (71): Where the label states that Penicillin G Sodium is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.
- **BACTERIAL ENDOTOXINS TEST** (85): Where the label states that Penicillin G Sodium is sterile or it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.01 USP Endotoxin Units/100 Penicillin G Units.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** (11)
 USP Endotoxin RS
 USP Penicillin G Potassium RS
 USP Penicillin G Sodium RS

Penicillin G Sodium for Injection

DEFINITION

Penicillin G Sodium for Injection is sterile Penicillin G Sodium or a sterile mixture of Penicillin G Sodium and NLT 4.0% and NMT 5.0% of Sodium Citrate, of which NMT 0.15% may be replaced by Citric Acid. It contains NLT 90.0% and NMT 120.0% of the labeled amount of penicillin G. In addition, where it contains Sodium Citrate, it has a potency of NLT 1420 and NMT 1667 Penicillin G Units/mg.

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHY

Solution A: Acetone, 0.1 M citric acid, and 0.1 M sodium citrate (2:1:1)

Standard solution: Prepare a solution containing the equivalent of 12,000 Penicillin G Units/mL from USP Penicillin G Potassium RS in *Solution A*.

Sample solution: Nominally 12,000 Penicillin G Units/mL from Penicillin G Sodium for Injection in *Solution A*

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 μ L

Developing solvent system: Toluene, dioxane, and glacial acetic acid (90:25:4)

Spray reagent 1: Starch TS

Spray reagent 2: Iodine TS diluted 1 in 10 with water

Analysis

Samples: *Standard solution* and *Sample solution*

Place the plate in a suitable chromatographic chamber. Develop the chromatogram using the *Developing sol-*

vent system until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow to air-dry. Spray the plate with *Spray reagent 1* followed by *Spray reagent 2*. Penicillin G appears as a white spot on a purple background.

Acceptance criteria: The R_f value of the penicillin G spot from the *Sample solution* corresponds to that from the *Standard solution*.

ASSAY

• PROCEDURE

Solution A: 0.01 M monobasic potassium phosphate

Mobile phase: Methanol and *Solution A* (40:60)

System suitability solution: 0.1 mg/mL each of USP Penicillin G Potassium RS and 2-phenylacetamide in water

Standard solution: 0.1 mg/mL of USP Penicillin G Potassium RS in water. Shake as needed to dissolve. This solution contains about 160 Penicillin G Units/mL.

Sample solution 1 (where it is represented as being in a single-dose container): Constitute Penicillin G Sodium for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a hypodermic needle and syringe, and dilute with water to obtain a solution containing nominally 160 Penicillin G Units/mL.

Sample solution 2 (where the label states the quantity of penicillin G in a given volume of constituted solution): Constitute Penicillin G Sodium for Injection as directed in the labeling. Dilute a suitable aliquot of the constituted solution with water to obtain a solution containing nominally 160 Penicillin G Units/mL.

Sample solution 3 (where it contains sodium citrate): Transfer about 50 mg of the Penicillin G Sodium for Injection to a 500-mL volumetric flask, add about 400 mL of water, and shake to dissolve. Dilute with water to volume, and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 2-phenylacetamide and penicillin G are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between 2-phenylacetamide and penicillin G, *System suitability solution*

Column efficiency: NLT 1000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution 1*, *Sample solution 2*, or *Sample solution 3*

Perform the Assay on 10 containers where it is represented as being in a single-dose container and, if necessary, on 10 containers where the label states the quantity of penicillin G in a given volume of constituted solution. Use the individual results to determine the *Uniformity of Dosage Units* and the average of the results as the Assay value.

Calculate the percentage of the labeled amount of penicillin G in the container or in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

- r_U = peak response from *Sample solution 1* or *Sample solution 2*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of *Sample solution 1* or *Sample solution 2* (Penicillin G Units/mL)
 P = potency of penicillin G in USP Penicillin G Potassium RS (Penicillin G Units/mg)

Calculate the potency, in Penicillin G Units/mg, in the portion of Penicillin G Sodium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

- r_U = peak response from *Sample solution 3*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Penicillin G Potassium RS in the *Standard solution* (mg/mL)
 C_U = concentration of Penicillin G Sodium for Injection in *Sample solution 3* (mg/mL)
 P = potency of penicillin G in USP Penicillin G Potassium RS (Penicillin G Units/mg)

Acceptance criteria: 90.0%–120.0% of the labeled amount of penicillin G. Where Penicillin G Sodium for Injection contains sodium citrate, it has a potency of NLT 1420 and NMT 1667 Penicillin G Units/mg.

PERFORMANCE TESTS

- UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements. Perform the Assay on 10 containers where it is represented as being in a single-dose container and, if necessary, on 10 containers where the label states the quantity of penicillin G in a given volume of constituted solution. Use the individual results to determine the *Uniformity of Dosage Units* and the average of the results as the Assay value.

SPECIFIC TESTS

- INJECTIONS AND IMPLANTED DRUG PRODUCTS (1), Specific Tests, Completeness and clarity of solutions:** At the time of use, it meets the requirements.
- CRYSTALLINITY (695):** Meets the requirements
- STERILITY TESTS (71):** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.01 USP Endotoxin Units/100 Penicillin G Units.
- PH (791)**
Sample solution: A solution containing 60 mg/mL
Acceptance criteria: 5.0–7.5. Where it is labeled as containing sodium citrate, the pH is between 6.0 and 7.5.
- LOSS ON DRYING (731)**
Sample: 100 mg of Penicillin G Sodium for Injection
Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at a pressure NMT 5 mm of mercury at 60° for 3 h.
Acceptance criteria: NMT 1.5%
- PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- LABELING (7), Labels and Labeling for Injectable Products:** Meets the requirements

ADDITIONAL REQUIREMENTS

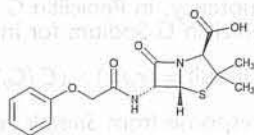
Change to read:

- PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

• **USP REFERENCE STANDARDS (11)**

- USP Endotoxin RS
USP Penicillin G Potassium RS

Penicillin V



$C_{16}H_{18}N_2O_5S$ 350.39
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-, [2S-(2 α ,5 α ,6 β)]-; (2S,5R,6R)-3,3-Dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid [87-08-1].

DEFINITION

Penicillin V has a potency of NLT 1525 and NMT 1780 Penicillin V Units/mg.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
Sample: Do not dry.
Acceptance criteria: Meets the requirements

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, glacial acetic acid, and water (350: 5.75: 650)

System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in Mobile phase

Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in Mobile phase

Sample solution: 2.5 mg/mL of Penicillin V in Mobile phase

Chromatographic system
(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm \times 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: System suitability solution and Standard solution

[NOTE—The relative retention times for *p*-hydroxyphenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between penicillin G and penicillin V, System suitability solution

Column efficiency: NLT 1800 theoretical plates, System suitability solution

Relative standard deviation: NMT 1.0% for penicillin V potassium, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the potency of penicillin V potassium, in Penicillin V Units/mg, in the portion of Penicillin V taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P$$

r_u = sum of the peak responses of *p*-hydroxyphenicillin V and penicillin V from the Sample solution

r_s = sum of the peak responses of *p*-hydroxyphenicillin V and penicillin V from the Standard solution

C_s = concentration of USP Penicillin V Potassium RS in the Standard solution (mg/mL)

C_u = concentration of Penicillin V in the Sample solution (mg/mL)

P = potency of penicillin V in USP Penicillin V Potassium RS (Penicillin V Units/mg)

Acceptance criteria: 1525–1780 Penicillin V Units/mg

IMPURITIES

• **LIMIT OF PHENOXYACETIC ACID**

Mobile phase: Acetonitrile, glacial acetic acid, and water (35:1:65)

Diluent: pH 6.6 phosphate buffer (see Reagents, Indicators, and Solutions—Buffer Solutions)

Standard solution: 0.1 mg/mL of phenoxyacetic acid in Diluent

Sample solution: 20.0 mg/mL of Penicillin V in Diluent. Use this solution on the day prepared.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of phenoxyacetic acid in the portion of Penicillin V taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak area of phenoxyacetic acid from the Sample solution

r_s = peak area of phenoxyacetic acid from the Standard solution

C_s = concentration of phenoxyacetic acid in the Standard solution (mg/mL)

C_u = concentration of Penicillin V in the Sample solution (mg/mL)

Acceptance criteria: NMT 0.5%

• **LIMIT OF *p*-HYDROXYPENICILLIN V**

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system and System suitability: Proceed as directed in the Assay.

Analysis

Sample: Sample solution

Calculate the percentage of *p*-hydroxyphenicillin V in the portion of Penicillin V taken:

$$\text{Result} = (r_u/r_T) \times 100$$

r_u = peak response of *p*-hydroxyphenicillin V from the Sample solution

r_T = sum of the peak responses of *p*-hydroxyphenicillin V and penicillin V

Acceptance criteria: NMT 5.0%

SPECIFIC TESTS

- **CRYSTALLINITY (695):** Meets the requirements

- **PH (791)**

Sample solution: Prepare a suspension containing 30 mg/mL of Penicillin V in water

Acceptance criteria: 2.5–4.0

- **WATER DETERMINATION** (921), *Method I*: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **LABELING**: Label it to indicate that it is to be used in the manufacture of nonparenteral drugs only.
- **USP REFERENCE STANDARDS** (11)
 - USP Penicillin G Potassium RS
 - USP Penicillin V RS
 - USP Penicillin V Potassium RS

Penicillin V for Oral Suspension

DEFINITION

Penicillin V for Oral Suspension is a dry mixture of Penicillin V with or without one or more suitable buffers, colors, flavors, and suspending agents. It contains NLT 90.0% and NMT 120.0% of the labeled number of Penicillin V Units when constituted as directed.

IDENTIFICATION

- **A.** The retention time of the penicillin V peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile, glacial acetic acid, and water (350: 5.75: 650)

System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in *Mobile phase*

Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*

Sample solution: Constitute Penicillin V for Oral Suspension as directed in the labeling. Transfer a suitable aliquot, freshly mixed and free from air bubbles, and containing about 400,000 Penicillin V Units to a suitable volumetric flask. Dilute with *Mobile phase* to volume, and mix to obtain a solution containing nominally about 4000 Penicillin V Units/mL. Pass a portion of this solution through a suitable filter of 0.5- μ m or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm \times 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for *p*-hydroxypenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between penicillin G and penicillin V, *System suitability solution*

Column efficiency: NLT 1800 theoretical plates for penicillin V, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled number of Penicillin V Units in the portion of Penicillin V for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Sample solution*

r_S = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Standard solution*

C_S = concentration of USP Penicillin V Potassium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of penicillin V in the *Sample solution* (Penicillin V Units/mL)

P = potency of penicillin V in USP Penicillin V Potassium RS (Penicillin V Units/mg)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905)

For solids packaged in single-unit containers: Meets the requirements

- **DELIVERABLE VOLUME** (698): Meets the requirements

SPECIFIC TESTS

- **PH** (791)

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 2.0–4.0

- **WATER DETERMINATION** (921), *Method I*: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **LABELING**: It may be labeled in terms of the weight of penicillin V contained therein, in addition to or instead of Units, on the basis that 1600 Penicillin V Units are equivalent to 1 mg of penicillin V.
- **USP REFERENCE STANDARDS** (11)
 - USP Penicillin G Potassium RS
 - USP Penicillin V RS
 - USP Penicillin V Potassium RS

Penicillin V Tablets

DEFINITION

Penicillin V Tablets contain NLT 90.0% and NMT 120.0% of the labeled number of Penicillin V Units.

IDENTIFICATION

- **A.** The retention time of the penicillin V peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile, glacial acetic acid, and water (350: 5.75: 650)

System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in *Mobile phase*

Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*

Sample solution: Nominally 4000 Penicillin V Units/mL, in *Mobile phase* dissolved from finely powdered Tablets (NLT 20). Shake for about 5 min. Pass a portion of the solution through a suitable filter of 0.5- μ m or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm \times 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for *p*-hydroxypenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between penicillin G and penicillin V, *System suitability solution*

Column efficiency: NLT 1800 theoretical plates, *System suitability solution*

Relative standard deviation: NMT 1.0% for penicillin V potassium, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled number of Penicillin V Units in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times 100$$

r_u = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Sample solution*

r_s = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Standard solution*

C_s = concentration of USP Penicillin V Potassium RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of penicillin V in the *Sample solution* (Penicillin V Units/mL)

P = potency of penicillin V in USP Penicillin V Potassium RS (Penicillin V Units/mg)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: USP Penicillin V Potassium RS in *Medium*

Sample solution: Sample per *Dissolution* (711). Dilute with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of penicillin V ($C_{16}H_{18}N_2O_5S$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

SPECIFIC TESTS

• WATER DETERMINATION (921), *Method I*: NMT 3.0%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in tight containers.

• LABELING:

The Tablets may be labeled in terms of the weight of penicillin V contained therein, in addition to or instead of Units, on the basis that 1600 Penicillin V Units are equivalent to 1 mg of penicillin V.

• USP REFERENCE STANDARDS (11)

USP Penicillin G Potassium RS

USP Penicillin V RS

USP Penicillin V Potassium RS

6 β], compd. with *N,N'*-bis(phenylmethyl)-1,2-ethanediamine (2:1).

(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with *N,N'*-dibenzylethylenediamine (2:1) [5928-84-7].

Tetrahedrate 1013.21 [63690-57-3].

» Penicillin V Benzathine has a potency of not less than 1060 and not more than 1240 Penicillin V Units per mg.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Penicillin V Potassium RS

Crystallinity (695): meets the requirements.

pH (791): between 4.0 and 6.5, in a suspension containing about 30 mg per mL.

Water Determination, Method I (921): between 5.0% and 8.0%.

Penicillin V content—Transfer about 40 mg, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Concomitantly determine the absorbances of this solution and of a similarly prepared Standard solution prepared with about 30 mg of USP Penicillin V Potassium RS at the wavelength of maximum absorbance at about 276 nm. Determine the percentage of penicillin V taken by the formula:

$$P(a_u / a_s)$$

in which P is the percentage content of penicillin V in the USP Penicillin V Potassium RS, and a_u and a_s are the absorptivities of the solution of the specimen and the Standard solution, respectively: between 62.3% and 72.5% is found.

Assay—

Standard preparation—Prepare as directed for *Standard preparation* under *Iodometric Assay—Antibiotics* (425), using USP Penicillin V Potassium RS.

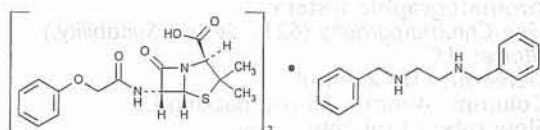
Assay preparations—Dissolve a quantity of Penicillin V Benzathine in 1.0 N sodium hydroxide to obtain a solution containing 2000 Penicillin V Units per mL. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask, and use as the *Assay preparation* for *Inactivation and titration*. Dilute a quantity of Penicillin V Benzathine quantitatively with water to obtain a suspension containing 2000 Penicillin V Units per mL. Pipet 2 mL of this suspension into a glass-stoppered, 125-mL conical flask, and use as the *Assay preparation* for the *Blank determination*.

Procedure—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425), except in the *Inactivation and titration* of the specimen to omit the addition of 2.0 mL of 1.0 N sodium hydroxide. Calculate the potency, in Penicillin V Units per mg, in the Penicillin V Benzathine taken by the formula:

$$(F)(B - I) / (2D)$$

in which D is the concentration, in mg per mL, of the *Assay preparation* for *Inactivation and titration* on the basis of the weight of Penicillin V Benzathine taken and the extent of dilution.

Penicillin V Benzathine



$(C_{16}H_{18}N_2O_5S)_2 \cdot C_{16}H_{20}N_2$ 941.12

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(2-phenoxyacetyl)amino]-, [2*S*-(2 α ,5 α ,

Penicillin V Benzathine Oral Suspension

» Penicillin V Benzathine Oral Suspension contains not less than 90.0 percent and not more than 120.0 percent of the labeled number of

Penicillin V Units per mL. It contains one or more suitable buffers, colors, dispersants, flavors, and preservatives.

Packaging and storage—Preserve in tight containers, and store in a refrigerator.

Labeling—It may be labeled in terms of the weight of penicillin V contained therein, in addition to or instead of Units, on the basis that 1600 Penicillin V Units are equivalent to 1 mg of penicillin V.

USP Reference standards (11)—

USP Penicillin V Potassium RS

Uniformity of dosage units (905)—

FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698): meets the requirements.

pH (791): between 6.0 and 7.0.

Assay—

Standard preparation—Prepare as directed for *Standard preparation under Iodometric Assay—Antibiotics* (425), using USP Penicillin V Potassium RS.

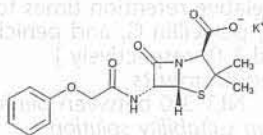
Assay preparations—Dilute an accurately measured volume of Oral Suspension, freshly mixed and free from air bubbles, quantitatively with 1.0 N sodium hydroxide to obtain a solution containing 2000 Penicillin V Units per mL. Pipet 2 mL of this solution into a glass-stoppered, 125-mL conical flask, and use as the *Assay preparation for Inactivation and titration*. Dilute an accurately measured volume of Oral Suspension quantitatively with water to obtain a suspension containing 2000 Penicillin V Units per mL. Pipet 2 mL of this suspension into a glass-stoppered, 125-mL conical flask, and use as the *Assay preparation for the Blank determination*.

Procedure—Proceed as directed for *Procedure under Iodometric Assay—Antibiotics* (425), except in the *Inactivation and titration* to omit the addition of 2.0 mL of 1.0 N sodium hydroxide. Calculate the quantity, in Penicillin V Units, in each mL of the Oral Suspension taken by the formula:

$$(T/2D)(B - I)$$

in which *T* is the labeled quantity, in Penicillin V Units per mL, in the Oral Suspension, and *D* is the concentration, in Penicillin V Units per mL, in the *Assay preparation for Inactivation and titration* on the basis of the volume of Oral Suspension taken and the extent of dilution.

Penicillin V Potassium



$C_{16}H_{17}KN_2O_5S$ 388.48
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenoxymethyl)amino]-, monopotassium salt, [2S-(2 α ,5 α ,6 β)]-;
Monopotassium (2S,5R,6R)-3,3-dimethyl-7-oxo-6-(2-phenoxymethylamino)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [132-98-9].

DEFINITION

Penicillin V Potassium has a potency of NLT 1380 and NMT 1610 Penicillin V Units/mg.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the penicillin V peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **C.**

Diluent: Glycerin and water (25:14)

Solution A: 106 mg/mL of sodium carbonate in water

Solution B: 120 mg/mL of sodium sulfide in *Diluent*, prepared as follows. Dissolve sodium sulfide in *Diluent*, using about 45% of the final volume and heat. Allow to cool, and dilute with *Diluent* to the final volume.

Solution C: 150 mg/mL of tartaric acid in water

Sample solution: 0.1 g of Penicillin V Potassium in 2 mL of water

Analysis

Part 1: Add 1 mL of *Solution A* to the *Sample solution* and heat.

Part 2: To the hot solution from *Part 1* add 0.05 mL of *Solution B*.

Part 3: Cool the mixture from *Part 2* in iced water and add 2 mL of *Solution C*. Allow to stand.

Acceptance criteria: Meets the requirements for *Parts 1, 2, and 3*

Part 1: No precipitate is formed.

Part 2: No precipitate is formed.

Part 3: A white precipitate is formed.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile, glacial acetic acid, and water (350:5.75:650)

System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in *Mobile phase*

Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*

Sample solution: 2.5 mg/mL of Penicillin V Potassium in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; 10- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for *p*-hydroxyphenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between penicillin G and penicillin V, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the potency of penicillin V potassium, in Penicillin V Units/mg, in the portion of Penicillin V Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = sum of the *p*-hydroxyphenicillin V and penicillin V peak responses from the *Sample solution*

r_S = sum of the *p*-hydroxyphenicillin V and penicillin V peak responses from the *Standard solution*

C_S = concentration of USP Penicillin V Potassium RS in the *Standard solution* (mg/mL)

C_U = concentration of Penicillin V Potassium in the *Sample solution* (mg/mL)

P = potency of USP Penicillin V Potassium RS (Penicillin V Units/mg)

Acceptance criteria: 1380–1610 Penicillin V Units/mg

IMPURITIES

• LIMIT OF PHENOXYACETIC ACID

Mobile phase: Acetonitrile, glacial acetic acid, and water (35:1:65)

Diluent: pH 6.6 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)

Standard solution: 0.1 mg/mL of phenoxyacetic acid in Diluent

Sample solution: 20 mg/mL of Penicillin V Potassium in Diluent. Use this solution on the day prepared.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of phenoxyacetic acid in the portion of Penicillin V Potassium taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = phenoxyacetic acid peak response from the Sample solution

r_s = phenoxyacetic acid peak response from the Standard solution

C_s = concentration of phenoxyacetic acid in the Standard solution (mg/mL)

C_u = concentration of Penicillin V Potassium in the Sample solution (mg/mL)

Acceptance criteria: NMT 0.5%

• LIMIT OF *p*-HYDROXYPENICILLIN V

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Sample: Sample solution

Calculate the percentage of *p*-hydroxyphenicillin V in the portion of Penicillin V Potassium taken:

$$\text{Result} = (r_u/r_T) \times 100$$

r_u = *p*-hydroxyphenicillin V peak response from the Sample solution

r_T = sum of the *p*-hydroxyphenicillin V and penicillin V peak responses from the Sample solution

Acceptance criteria: NMT 5.0%

SPECIFIC TESTS

• OPTICAL ROTATION (781S), *Specific Rotation*

Sample solution: 10 mg/mL of Penicillin V Potassium in carbon dioxide-free water

Acceptance criteria: +220° to +235°

• CRYSTALLINITY (695): Meets the requirements

• PH (791)

Sample solution: 30 mg/mL of Penicillin V Potassium in water

Acceptance criteria: 4.0–7.5

• LOSS ON DRYING (731)

Sample: 100 mg of Penicillin V Potassium

Analysis: Dry the Sample in a capillary-stoppered bottle under vacuum at 60° for 3 h.

Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label it to indicate that it is to be used in the manufacture of nonparenteral drugs only.

• **USP REFERENCE STANDARDS (11)**

USP Penicillin G Potassium RS

USP Penicillin V Potassium RS

Penicillin V Potassium for Oral Solution

DEFINITION

Penicillin V Potassium for Oral Solution is a dry mixture of Penicillin V Potassium with or without one or more suitable buffers, colors, flavors, preservatives, and suspending agents. It contains NLT 90.0% and NMT 135.0% of the labeled number of Penicillin V Units when constituted as directed.

IDENTIFICATION

• **A.** The retention time of the penicillin V peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile, glacial acetic acid, and water (35:5.75:650)

System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in Mobile phase

Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in Mobile phase

Sample solution: Constitute Penicillin V Potassium for Oral Solution as directed in the labeling. Transfer a suitable aliquot containing nominally 400,000 Penicillin V Units to a suitable volumetric flask. Dilute with Mobile phase to volume, and mix to obtain a solution containing nominally 4000 Penicillin V Units/mL. Pass a portion of this solution through a suitable filter of 0.5-μm or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: System suitability solution and Standard solution

[NOTE—The relative retention times for *p*-hydroxyphenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between penicillin G and penicillin V, System suitability solution

Column efficiency: NLT 1800 theoretical plates, System suitability solution

Relative standard deviation: NMT 1.0%, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled number of Penicillin V Units in the portion of Penicillin V Potassium for Oral Solution taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times 100$$

- r_U = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Sample solution*
 r_S = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Standard solution*
 C_S = concentration of USP Penicillin V Potassium RS in the *Standard solution* (mg/mL)
 C_U = nominal concentration of penicillin V in the *Sample solution* (Penicillin V Units/mL)
 P = potency of penicillin V in USP Penicillin V Potassium RS (Penicillin V Units/mg)
 Acceptance criteria: 90.0%–135.0%

PERFORMANCE TESTS• **UNIFORMITY OF DOSAGE UNITS** (905)

For solids packaged in single-unit containers: Meets the requirements

• **DELIVERABLE VOLUME** (698): Meets the requirements**SPECIFIC TESTS**• **PH** (791)

Sample solution: Constitute as directed in the labeling.
 Acceptance criteria: 5.0–7.5

• **WATER DETERMINATION** (921), *Method I*: NMT 1.0%**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **LABELING**: It may be labeled in terms of the weight of penicillin V contained therein, in addition to or instead of Units, on the basis that 1600 Penicillin V Units are equivalent to 1 mg of penicillin V.
- **USP REFERENCE STANDARDS** (11)
 USP Penicillin G Potassium RS
 USP Penicillin V Potassium RS

Penicillin V Potassium Tablets**DEFINITION**

Penicillin V Potassium Tablets contain NLT 90.0% and NMT 120.0% of the labeled number of Penicillin V Units.

IDENTIFICATION

- **A**. The retention time of the penicillin V peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Mobile phase: Acetonitrile, glacial acetic acid, and water (350:5.75:650)

System suitability solution: 2.5 mg/mL each of USP Penicillin G Potassium RS and USP Penicillin V Potassium RS in *Mobile phase*

Standard solution: 2.5 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*

Sample solution: Transfer a portion of finely powdered Tablets (NLT 20), containing nominally about 400,000 Penicillin V Units, to a suitable volumetric flask. Dilute with *Mobile phase* to volume, mix to obtain a solution containing nominally about 4000 Penicillin V Units/mL and shake for about 5 min. Pass a portion of this solution through a suitable filter of 0.5-μm or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for *p*-hydroxypenicillin V, penicillin G, and penicillin V are about 0.4, 0.8, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between penicillin G and penicillin V, *System suitability solution*

Column efficiency: NLT 1800 theoretical plates, *System suitability solution*

Relative standard deviation: NMT 1.0% for penicillin V potassium, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled number of Penicillin V Units in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Sample solution*

r_S = sum of the peak responses of *p*-hydroxypenicillin V and penicillin V from the *Standard solution*

C_S = concentration of USP Penicillin V Potassium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of penicillin V in the *Sample solution* (Penicillin V Units/mL)

P = potency of penicillin V in USP Penicillin V Potassium RS (Penicillin V Units/mg)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)

Medium: pH 6.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: USP Penicillin V Potassium RS in *Medium*

Sample solution: Sample per *Dissolution* (711). Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

Analysis: Determine the percentage of the labeled number of Penicillin V Units dissolved by a suitable validated spectrophotometric analysis of a filtered portion of the solution under test.

Tolerances: NLT 75% (Q) of the labeled number of Penicillin V Units is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

SPECIFIC TESTS• **LOSS ON DRYING** (731)

Sample: 100 mg

Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.

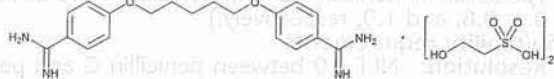
Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **LABELING**: Label the chewable Tablets to indicate that they are to be chewed before swallowing. The Tablets may be labeled in terms of the weight of penicillin V contained therein, in addition to or instead of Units, on the basis that 1600 Penicillin V Units are equivalent to 1 mg of penicillin V.

- **USP REFERENCE STANDARDS (11)**
USP Penicillin G Potassium RS
USP Penicillin V Potassium RS

Pentamidine Isethionate



$C_{19}H_{24}N_4O_2 \cdot (C_2H_6O_4S)_2$ 592.68
Ethanesulfonic acid, 2-hydroxy-, compd. with 4,4'-[1,5-pentanediyldis(oxy)]bis[benzenecarboximidamide]; 4,4'-(Pentane-1,5-diylbis(oxy))dibenzimidamide bis(2-hydroxyethanesulfonate) [140-64-7].

DEFINITION

Pentamidine Isethionate contains NLT 98.5% and NMT 101.5% of $C_{19}H_{24}N_4O_2 \cdot (C_2H_6O_4S)_2$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B. OXYGEN-FLASK COMBUSTION (471)**
Barium chloride solution: 60 mg/mL of barium chloride in water
Analysis: Burn 150 mg, using 10 mL of 3% hydrogen peroxide as the absorbing liquid. When the process is complete, acidify with 1 mL of diluted hydrochloric acid, and add 1 mL of the Barium chloride solution. Acceptance criteria: A white precipitate is formed.
- **C.** The retention time of the pentamidine isethionate peak of the Sample solution corresponds to that of the Standard solution, as obtained in the test for Organic Impurities.

ASSAY

- **PROCEDURE**
Sample solution: 5 mg/mL in dimethylformamide. Add 0.25 mL of thymol blue TS.
Analysis: Titrate under a stream of nitrogen with 0.1 M tetrabutylammonium hydroxide VS, determining the endpoint until the color changes to intense blue. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 M tetrabutylammonium hydroxide is equivalent to 29.63 mg of $C_{19}H_{24}N_4O_2 \cdot (C_2H_6O_4S)_2$.
Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

Inorganic Impurities

Delete the following:

- **HEAVY METALS, Method I (231):** NMT 20 ppm (Official 1-Jan-2018)

RESIDUE ON IGNITION (281)

Acceptance criteria: NMT 0.1% on a 1-g sample

Organic Impurities

PROCEDURE

Buffer: 30 mg/mL of ammonium acetate in water, adjusted with triethylamine to a pH of 7.5
Mobile phase: Methanol and Buffer (65:35)
System suitability solution: Prepare 40.0 mL of a 2.5 mg/mL solution of USP Pentamidine Isethionate RS in water. Adjust with 0.2 M sodium hydroxide to a pH of 10.5, and boil under reflux for 20 min. Cool, and dilute with water to 50.0 mL. Transfer quantitatively 1 mL of this solution to a 50-mL volumetric flask, and dilute with Mobile phase to volume.

Standard solution: 2 µg/mL of USP Pentamidine Isethionate RS in Mobile phase

Sample solution: 1.0 mg/mL of Pentamidine Isethionate in Mobile phase. [NOTE—It must be demonstrated that the final product does not contain a detectable amount of alkyl 2-hydroxyethanesulphonates, a potential in-process impurity.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

Run time: 3.5 times the retention time of pentamidine

System suitability

Sample: System suitability solution

Suitability requirements

Resolution: NLT 2 between the two major peaks.

[NOTE—The chromatogram shows two major peaks.]

Analysis

Samples: Standard solution and Sample solution

Acceptance criteria

Individual impurities: NMT 0.4%. [NOTE—Exclude any other peak producing a response of less than 0.02%.]

Total impurities: NMT 0.7%

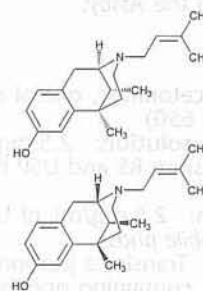
SPECIFIC TESTS

- **PH (791):** 4.5–6.5, in a carbon dioxide-free aqueous solution containing 50 mg/mL of Pentamidine Isethionate
- **LOSS ON DRYING (731):** Dry at 105°: it loses NMT 4.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Pentamidine Isethionate RS
 $C_{19}H_{24}N_4O_2 \cdot (C_2H_6O_4S)_2$

Pentazocine



$C_{19}H_{27}NO$ 285.42
2,6-Methano-3-benzazocin-8-ol, 1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-, (2α,6α,11R)-; (2R*,6R*,11R*)-1,2,3,4,5,6-Hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol [359-83-1].

DEFINITION

Pentazocine contains NLT 98.0% and NMT 101.5% of pentazocine ($C_{19}H_{27}NO$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)
Sample solution: 80 µg/mL in 0.01 N hydrochloric acid
Analytical wavelength: 278 nm
Acceptance criteria: Absorptivities do not differ by more than 3.0%, calculated on the dried basis.

ASSAY

- **PROCEDURE**
Sample solution: Dissolve about 500 mg of Pentazocine in 50 mL of glacial acetic acid.
Analysis: Add 1 drop of crystal violet TS to the *Sample solution*, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 28.54 mg of pentazocine ($C_{19}H_{27}NO$).
Acceptance criteria: 98.0%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%
- **ORDINARY IMPURITIES** (466)
Standard solution: Methanol
Test solution: Methanol
Eluant: Chloroform, methanol, and isopropylamine (94:3:3)
Visualization: Heat the plate in an oven at 105° for 15 min. Cool, follow with visualization technique 17, and view under short-wavelength UV light.
Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** (741): 147°–158°, with slight darkening
- **LOSS ON DRYING** (731)
Analysis: Dry at a pressure not exceeding 5 mm of mercury at 60° to constant weight.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
USP Pentazocine RS

Pentazocine Hydrochloride

$C_{19}H_{27}NO \cdot HCl$ 321.88
2,6-Methano-3-benzazocin-8-ol, 1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-, hydrochloride, (2 α ,6 α ,11 R^*)-;
(2 R^* ,6 R^* ,11 R^*)-1,2,3,4,5,6-Hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol hydrochloride [64024-15-3].

DEFINITION

Pentazocine Hydrochloride contains NLT 98.0% and NMT 102.0% of pentazocine hydrochloride ($C_{19}H_{27}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION** (197U)
Sample solution: 80 µg/mL of pentazocine
Medium: 0.01 N hydrochloric acid
Analytical wavelength: 278 nm
Acceptance criteria: Absorptivities do not differ by more than 3.0%, calculated on the dried basis. [NOTE—The molecular weight of pentazocine ($C_{19}H_{27}NO$) is 285.43.]

- **B. IDENTIFICATION—ORGANIC NITROGENOUS BASES** (181)

Standard solution: Dissolve 50 mg of USP Pentazocine RS in 25 mL of 0.01 N hydrochloric acid in a separator, and use this in place of the *Standard solution* specified.

Acceptance criteria: Meets the requirements

- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): A solution (1 in 100) meets the requirements.

ASSAY

- **PROCEDURE**
Sample solution: Dissolve 650 mg of Pentazocine Hydrochloride in 50 mL of glacial acetic acid.
Analysis: To the *Sample solution* add 10 mL of mercuric acetate TS and 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 32.19 mg of pentazocine hydrochloride ($C_{19}H_{27}NO \cdot HCl$).
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%
- **ORDINARY IMPURITIES** (466)
Standard solution: Methanol, USP Pentazocine RS being used
Test solution: Methanol
Eluant: Chloroform, methanol, and isopropylamine (94:3:3)
Visualization: Heat the plate in an oven at 105° for 15 min. Cool, follow with visualization technique 17, and view under short-wavelength UV light.
Acceptance criteria: The total of any ordinary impurities observed does not exceed 1.0%.

SPECIFIC TESTS

- **LOSS ON DRYING** (731)
Analysis: Dry at a pressure not exceeding 5 mm of mercury at 100° to constant weight.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
USP Pentazocine RS

Pentazocine and Acetaminophen Tablets**DEFINITION**

Pentazocine and Acetaminophen Tablets contain an amount of Pentazocine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ($C_8H_9NO_2$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)
Diluent: Chloroform and methanol (1:1)
Standard solution A: 1 mg/mL of USP Pentazocine RS in *Diluent*
Standard solution B: 26 mg/mL of USP Acetaminophen RS in *Diluent*
Sample solution: Transfer a quantity of finely powdered Tablets, nominally equivalent to about 5 mg of pentazocine and 130 mg of acetaminophen, to a suitable flask. Add 5 mL of *Diluent*, shake, and allow the solids to settle. Use the supernatant.

Chromatographic system

Developing solvent system: Ethyl acetate, methanol, and formic acid (90:5:5)

Spray reagent: Dissolve 300 mg of platinum chloride in 100 mL of water and add 100 mL of potassium iodide solution (6 in 100).

Analysis: Evaporate the solvents in cool, circulating air. After developing and examining the spots, spray the plate with *Spray reagent*.

Acceptance criteria: The R_f values, size, and intensity of color of the two principal spots of the *Sample solution* correspond to those of *Standard solution A* and *Standard solution B*.

ASSAY**Change to read:**• **PENTAZOCINE**

Mobile phase: Chloroform, methanol, and isopropylamine (960:40:2)

Diluent: Methanol and 0.035 N sulfuric acid (1:1)

Standard stock solution: 0.5 mg/mL of USP

Pentazocine RS in *Diluent*

Standard solution: Transfer 10.0 mL of the *Standard stock solution* to a 125-mL separator. Add 30 mL of water and 5 mL of sodium carbonate solution (1:10). Extract with 60 mL of chloroform and pass the chloroform layer through filter paper, collecting the filtrate in a 100-mL volumetric flask. Dilute with chloroform to volume and mix.

Sample solution: Transfer an amount nominally equivalent to 25 mg of pentazocine, from NLT 20 finely powdered Tablets, to a 50-mL glass-stoppered cylinder. Add 50.0 mL of *Diluent* and shake intermittently for 15 min. Sonicate for about 2 min, allow the solids to settle, and transfer 10.0 mL of the supernatant to a 125-mL separator. [NOTE—Save the remainder of the supernatant for use in the Assay for *Acetaminophen*. Minimize the waiting period before this test is performed to prevent significant hydrolysis of acetaminophen to *p*-aminophenol.]

Add 30 mL of water and 5 mL of sodium carbonate solution (1:10) to the separator and mix. Extract with 60 mL of chloroform and pass the chloroform layer through filter paper, collecting the filtrate in a 100-mL volumetric flask. Dilute with chloroform to volume and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 10-μm packing L3

Flow rate: 1.2 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

▲*USP40*

Tailing factor: NMT 3.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pentazocine ($C_{19}H_{27}NO$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pentazocine from the *Sample solution*

r_S = peak response of pentazocine from the *Standard solution*

C_S = concentration of USP Pentazocine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pentazocine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

Change to read:• **ACETAMINOPHEN**

[NOTE—Minimize the time between the addition of the *Diluent* and the injection of the *Sample solution* to prevent significant hydrolysis of acetaminophen to *p*-aminophenol.]

Mobile phase: Chloroform, methanol, and isopropylamine (960:40:2)

Diluent: Methanol and 0.035 N sulfuric acid (1:1)

Standard stock solution: 13 mg/mL of USP Acetaminophen RS in *Diluent*

Standard solution: Dilute 2.0 mL of the *Standard stock solution* with ethyl acetate to 200 mL.

Sample solution: Dilute 2.0 mL of the supernatant reserved from the Assay for *Pentazocine* immediately with ethyl acetate to volume in a 200-mL volumetric flask to minimize hydrolysis of acetaminophen to *p*-aminophenol and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 10-μm packing L3

Flow rate: 1.4 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

▲*USP40*

Tailing factor: NMT 3.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of acetaminophen from the *Sample solution*

r_S = peak response of acetaminophen from the *Standard solution*

C_S = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **UNIFORMITY OF DOSAGE UNITS (905)****Procedure for content uniformity**

Diluent: Acetonitrile and 0.035 N sulfuric acid (6:4)

Mobile phase: Tetrahydrofuran, phosphoric acid, and 0.005 M monobasic sodium phosphate (50:1:950)

Pentazocine standard stock solution: 0.25 mg/mL of USP Pentazocine RS in *Diluent*

System suitability stock solution: 0.325 mg/mL of USP Acetaminophen RS in *Diluent*

System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* to a 100-mL volumetric flask. Add 5.0 mL of the *Pentazocine standard stock solution*, dilute with *Mobile phase* to volume, and mix.

Standard solution: Transfer a quantity of USP Acetaminophen RS to a suitable volumetric flask. Add a sufficient volume of *Pentazocine standard stock solution* and mix to dissolve the acetaminophen. Dilute with *Mobile phase* to volume. Mix to obtain known concen-

trations of 0.0125 and 0.325 mg/mL of pentazocine and acetaminophen, respectively.

Sample stock solution: Transfer 1 Tablet to a 100-mL volumetric flask, add 50 mL of *Diluent*, and sonicate for 30 min. Dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a paper filter, covering the funnel with a watch glass and discarding the first few mL of the filtrate.

Sample solution: Dilute 5.0 mL of the *Sample stock solution* with *Mobile phase* to 100 mL and pass this solution through a membrane filter of 0.5- μ m or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 9.4-mm \times 10-cm; 5- μ m packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μ L

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for acetaminophen and pentazocine are 0.2 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 7 between pentazocine and acetaminophen

Relative standard deviation: NMT 2.0% for the pentazocine and acetaminophen peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and acetaminophen ($C_8H_9NO_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of pentazocine or acetaminophen from the *Sample solution*
 r_S = peak response of pentazocine or acetaminophen from the *Standard solution*
 C_S = concentration of the appropriate USP Reference Standard (USP Pentazocine RS or USP Acetaminophen RS) in the *Standard solution* (mg/mL)
 C_U = nominal concentration of pentazocine or acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

IMPURITIES

Add the following:

- ▲ **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** (227): Meet the requirements. ▲ USP40

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
 USP Acetaminophen RS
 USP Pentazocine RS

Pentazocine and Aspirin Tablets

DEFINITION

Pentazocine and Aspirin Tablets contain an amount of Pentazocine Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and NLT 90.0% and NMT 110.0% of the labeled amount of aspirin ($C_9H_8O_4$).

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

Diluent: Chloroform and methanol (1:1)

Standard solution A: 2.5 mg/mL of USP Pentazocine RS in *Diluent*

Standard solution B: 65 mg/mL of USP Aspirin RS in *Diluent*

Sample solution: Shake a quantity of finely powdered Tablets, nominally equivalent to about 25 mg of pentazocine and 650 mg of aspirin, with 10 mL of *Diluent* in an ultrasonic bath for 2 min. Allow the solids to settle.

Chromatographic system

Developing solvent system: Ethyl acetate, methanol, and formic acid (90:5:5)

Spray reagent: Iodoplatinate spray reagent. Dissolve 300 mg of platinum chloride in 100 mL of water, and add 100 mL of potassium iodide solution (6 in 100).

Analysis: Evaporate the solvents from the spots in warm circulating air. Place the plate in the developing chamber, and after developing the plate, remove it, and mark the solvent front. Evaporate the solvents thoroughly in warm circulating air, and examine the plate under short-wavelength UV light. Expose the plate to iodine vapor for about 5 min, and observe. Then spray the plate with *Spray reagent*.

Acceptance criteria: Two principal spots from the *Sample solution* correspond in R_f values, size, and intensity of color with the pentazocine spot of *Standard solution A* and the aspirin spot of *Standard solution B*, respectively.

ASSAY

• PROCEDURE

Diluent A: Methanol and water (1:1)

Diluent B: Methanol and 6 N hydrochloric acid (1:1)

Diluent C: Water, methanol, and 6 N hydrochloric acid (6:1:1)

Chromatographic column: Use a 200-mm tube consisting of about a 90-mm length of 22-mm tubing fused to about a 100-mm length of 5-mm tubing having a stopcock at the bottom of this section. Place a pledget of glass wool at the bottom of the 5-mm portion just above the stopcock. Transfer a suitable quantity of sulfonic acid cation-exchange resin to a beaker, and wash three times with water, discarding the water wash each time by decantation. Cover the resin with *Diluent B*, and allow to stand for 1 h. Decant the acid wash; if it is colored yellow or orange, repeat this step until the wash is almost colorless. Then wash the resin by repeated 15-min soakings in *Diluent A* followed by decantation until the wash is neutral to wide-range indicator paper. Fill the tube to a height of 100 mm with slurry of the washed resin in *Diluent A*. Wash the column with 25 mL of *Diluent A*.

Standard solution A: 18 μ g/mL of USP Salicylic Acid RS in 0.1 N sodium hydroxide

Standard solution B: 62.5 μ g/mL of USP Pentazocine RS in *Diluent C*

Sample stock solution A: Transfer a portion of fine powder from NLT 20 freshly powdered Tablets, nominally equivalent to about 25 mg of pentazocine and 650 mg of aspirin from NLT 20 finely powdered Tablets, to a suitable 250-mL flask. Add 100.0 mL of *Diluent A*, and shake by mechanical means for 20 min. Centrifuge a suitable quantity for 5 min.

Sample stock solution B: Transfer 25.0 mL of the clear supernatant from *Sample stock solution A* to the prepared *Chromatographic column*, followed by five 10-mL portions of *Diluent A*, collecting the eluate in a 250-mL volumetric flask containing 10.0 mL of 2.5 N sodium hydroxide. Dilute with water to volume, and mix.

Sample solution A: Pipet 4 mL of *Sample stock solution B* into a 100-mL volumetric flask, and dilute with 0.1 N sodium hydroxide to volume, and mix.

Sample solution B: Pass through the column after *Sample stock solution B* five 5-mL portions of *Diluent B*, followed by 10 mL of water. Collect the eluate in a 100-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: 296 nm (maximum absorbance) for the analysis of aspirin; 278 nm (maximum absorbance) for the analysis of pentazocine

Cell: 1 cm

Blank: 0.1 N sodium hydroxide for the analysis of aspirin; *Diluent C* for the analysis of pentazocine

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Sample solution A*, and *Sample solution B*

Calculate the percentage of aspirin ($C_9H_8O_4$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance from *Sample solution A*

A_S = absorbance from *Standard solution A*

C_S = concentration of USP Salicylic Acid RS in *Standard solution A* ($\mu\text{g/mL}$)

C_U = nominal concentration of aspirin in *Sample solution A* ($\mu\text{g/mL}$)

M_{r1} = molecular weight of aspirin, 180.16

M_{r2} = molecular weight of salicylic acid, 138.12

Calculate the percentage of pentazocine ($C_{19}H_{27}NO$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance from *Sample solution B*

A_S = absorbance from *Standard solution B*

C_S = concentration of USP Pentazocine RS in *Standard solution B* ($\mu\text{g/mL}$)

C_U = nominal concentration of pentazocine in *Sample solution B* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–110.0% each of the labeled amounts of aspirin ($C_9H_8O_4$) and pentazocine ($C_{19}H_{27}NO$)

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 1: 80 rpm

Time: 30 min

Standard solution A: 15 $\mu\text{g/mL}$ of USP Salicylic Acid RS in 0.1 N sodium hydroxide

Standard solution B: 13 $\mu\text{g/mL}$ of USP Pentazocine RS in dilute glacial acetic acid (1 in 50)

Strongly basic, anion-exchange resin: Mix a suitable quantity of anion-exchange resin with 10 volumes of dilute glacial acetic acid (1 in 50), and shake for 20 min. Allow the resin to settle, and decant the supernatant. Repeat the acetic acid washing four more times. Wash with water until 5.0 mL of the water wash gives a negligible response when substituted for 5.0 mL of *Sample solution*, and carried through the *Analysis* for pentazocine.

Sample solution: To a suitable 50-mL flask add 0.4 g of *Strongly basic, anion-exchange resin* and 25 mL of the solution under test. Shake by mechanical means for 15 min. Allow to settle, and use the clear supernatant.

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 296 nm (maximum absorbance) for the analysis of aspirin; 408 nm (maximum absorbance) for the analysis of pentazocine

Cell: 1 cm

Blank: 0.1 N sodium hydroxide for the analysis of aspirin; the chloroform layer from the reagent blank for the analysis of pentazocine

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

For aspirin, transfer 1.0 mL of the *Sample solution* to a 25-mL volumetric flask containing 1.0 mL of sodium hydroxide solution (1 in 10), and swirl. Allow to stand for 10 min. Dilute with water to volume, and mix.

Determine the absorbances of the *Sample solution* and *Standard solution A* against the *Blank* for the analysis of aspirin.

Calculate the percentage of the labeled amount of aspirin ($C_9H_8O_4$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance from the *Sample solution*

A_S = absorbance from *Standard solution A*

C_S = concentration of USP Salicylic Acid RS in *Standard solution A* (mg/mL)

L = labeled amount of aspirin (mg/Tablet)

V = volume of *Medium*, 900 mL

M_{r1} = molecular weight of aspirin, 180.16

M_{r2} = molecular weight of salicylic acid, 138.12

For pentazocine, transfer 5.0-mL portions of the *Sample solution*, *Standard solution B*, and water to serve as the reagent blank into three separate 125-mL separators.

To each separator add 10 mL of a filtered solution (1 in 4000) of bromocresol purple in dilute glacial acetic acid (1 in 50) and 20.0 mL of chloroform. Insert the stopper, and shake gently for 1 min, accurately timed. Allow the layers to separate, and determine the absorbances of the clear chloroform layers from *Standard solution B* and the *Sample solution* against the chloroform layer from the reagent blank.

Calculate the percentage of the labeled amount of pentazocine ($C_{19}H_{27}NO$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

A_U = absorbance from the *Sample solution*

A_S = absorbance from *Standard solution B*

C_S = concentration of USP Pentazocine RS in *Standard solution B* (mg/mL)

L = labeled amount of pentazocine (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and NLT 70% (Q) of the labeled amount of aspirin ($C_9H_8O_4$) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

• NONASPIRIN SALICYLATES

Ferric chloride-urea reagent: To a mixture of 8 mL of ferric chloride solution (6 in 10) and 42 mL of 0.05 N hydrochloric acid, add 60 g of urea. Dissolve the urea by swirling and without the aid of heat, and adjust the resulting solution, if necessary, with 6 N hydrochloric acid to a pH of 3.2. Prepare on the day of use.

Standard stock solution: 150 $\mu\text{g/mL}$ of salicylic acid in chloroform

Standard solution: Pipet 5 mL of *Standard stock solution* into a 50-mL volumetric flask containing 10 mL of methanol, 2 drops of hydrochloric acid, and 10 mL of a solution (1 in 10) of glacial acetic acid in ether. Add chloroform to volume, and mix.

Sample solution

Insert a small pledget of glass wool above the stem constriction of a 20- × 2.5-cm chromatographic tube, and uniformly pack with a mixture of about 1 g of chromatographic siliceous earth and 0.5 mL of 5 M

phosphoric acid. Directly above this layer, pack a similar mixture of about 3 g of chromatographic siliceous earth and 2 mL of *Ferric chloride-urea reagent*. To a quantity nominally equivalent to 50 mg of aspirin from finely powdered Tablets add 10 mL of chloroform, stir for 3 min, and transfer to the chromatographic adsorption column with the aid of 5 mL of chloroform. Pass 50 mL of chloroform in several portions through the column, rinse the tip of the chromatographic tube with chloroform, and discard the eluate. If the purple zone reaches the bottom of the tube, discard the column, and repeat the test with a smaller quantity of powdered Tablets.

Elute the adsorbed salicylic acid into a 100-mL volumetric flask containing 20 mL of methanol and 4 drops of hydrochloric acid by passing two 10-mL portions of a solution (1 in 10) of glacial acetic acid in water-saturated ether, and then 30 mL of chloroform, through the column, and dilute the eluate with chloroform to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: 306 nm (maximum absorbance)

Cell: 1 cm

Analysis: Concomitantly determine the absorbances of both the *Sample solution* and *Standard solution*, using a solvent mixture of the same composition as that of the *Standard solution* as the blank.

Acceptance criteria: 3.0%; the absorbance of the *Sample solution* does not exceed that of the *Standard solution*, any necessary adjustment being made for having used a smaller sample.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP Aspirin RS
USP Pentazocine RS
USP Salicylic Acid RS

Pentazocine and Naloxone Tablets

DEFINITION

Pentazocine and Naloxone Tablets contain amounts of Pentazocine Hydrochloride and Naloxone Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amounts of pentazocine ($C_{19}H_{27}NO$) and naloxone ($C_{19}H_{21}NO_4$).

IDENTIFICATION

• **A.**

Diluent: Chloroform and methanol (1:1)

Standard solution A: 5.0 mg/mL of USP Pentazocine RS in *Diluent*

Standard solution B: 1.3 mg/mL of USP Naloxone RS in *Diluent*

Sample solution A: Crush 1 Tablet in 10 mL of *Diluent*. Sonicate for about 2 min, and filter.

Sample solution B: Evaporate 5 mL of *Sample solution A* to dryness on a steam bath under a stream of nitrogen. Dissolve the residue in 0.2 mL of *Diluent*.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L, *Standard solution A* and *Sample solution A*; 5 μ L, *Standard solution B* and *Sample solution B*

Developing solvent system: 1-Butanol, water, and glacial acetic acid (70:20:10)

Spray reagent: Folin-Ciocalteu Phenol TS followed by sodium hydroxide solution (1 in 10)

Analysis: Develop the chromatograms in the *Developing solvent system* until the solvent has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and dry under a current of warm air. Spray the plate with *Spray reagent*.

Acceptance criteria: *Sample solution A* and *Sample solution B* exhibit spots having the same R_f values and approximately the same sizes and shapes as their respective *Standard solutions*.

ASSAY

• PROCEDURE

Diluent: Methanol, water, and phosphoric acid (500:500:1)

Solution A: Prepare a filtered and degassed mixture by dissolving 675 mg of sodium 1-octanesulfonate and 426 mg of anhydrous dibasic sodium phosphate in 625 mL of water, and mix.

Mobile phase: Add 475 mL of methanol and 10 mL of phosphoric acid to *Solution A*.

Strong anion-exchange resin: Transfer 30 g of strong anion-exchange resin to a 250-mL beaker. Wash the resin with two 200-mL portions of water, decanting the water after each wash. Wash with two 200-mL portions of dilute glacial acetic acid (1 in 20), decanting the first wash, and filter with the aid of suction.

Standard stock solution: 0.2 mg/mL of USP Naloxone RS in *Diluent*

Standard solution: Transfer 100 mg of USP Pentazocine RS to a 50-mL volumetric flask. Dissolve in about 30 mL of *Diluent*. Add 5.0 mL of the *Standard stock solution*, and dilute with *Diluent* to volume.

Sample solution: Transfer an amount nominally equivalent to 100 mg of pentazocine from NLT 20 Tablets to a 100-mL volumetric flask, and add 50.0 mL of *Diluent*. Sonicate for 5 min, and shake intermittently for 15 min. Filter into a glass-stoppered conical flask. Add about 250 mg of *Strong anion-exchange resin*, and shake for 30 min.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 229 nm

Column: 4.6-mm \times 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for naloxone and pentazocine are about 0.3 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6 between pentazocine and naloxone

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and naloxone ($C_{19}H_{21}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pentazocine or naloxone from the *Sample solution*

r_S = peak response of pentazocine or naloxone from the *Standard solution*

C_S = concentration of the appropriate USP Pentazocine RS or USP Naloxone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pentazocine or naloxone in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0% of the labeled amounts of pentazocine ($C_{19}H_{27}NO$) and naloxone ($C_{19}H_{21}NO_4$)

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Detector: UV 279 nm (corrected for absorbance at 305 nm)

Standard solution: Dissolve a suitable amount of USP Pentazocine RS in a minimum volume of 0.1 N hydrochloric acid (about 25 mg/mL), diluting quantitatively and stepwise with water.

Sample solution: Filter portions of the solution under test, suitably diluted with *Medium*, if necessary.

Analysis: Determine the labeled amount of pentazocine ($C_{19}H_{27}NO$) dissolved in the *Sample solution* in comparison with the *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of pentazocine ($C_{19}H_{27}NO$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Diluent: Methanol, water, and phosphoric acid (500:500:1)

Solution A: Prepare a filtered and degassed mixture by dissolving 675 mg of sodium 1-octanesulfonate and 426 mg of anhydrous dibasic sodium phosphate in 625 mL of water, and mix.

Mobile phase: Add 475 mL of methanol and 10 mL of phosphoric acid to *Solution A*.

Strong anion-exchange resin: Transfer 30 g of strong anion-exchange resin to a 250-mL beaker. Wash the resin with two 200-mL portions of water, decanting the water after each wash. Wash with two 200-mL portions of dilute glacial acetic acid (1 in 20), decanting the first wash, and filter with the aid of suction.

Standard stock solution: 0.2 mg/mL of USP Naloxone RS in *Diluent*

Standard solution: Transfer 100 mg of USP Pentazocine RS to a 50-mL volumetric flask. Dissolve in about 30 mL of *Diluent*. Add 5.0 mL of the *Standard stock solution*, and dilute with *Diluent* to volume.

Sample solution: Transfer 1 Tablet to a 25-mL glass-stoppered cylinder. Add 25.0 mL of *Diluent*. Sonicate for 10 min, and shake intermittently for 15 min. Filter into a glass-stoppered conical flask. Add about 125 mg of *Strong anion-exchange resin*, and shake for 30 min.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 229 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for naloxone and pentazocine are about 0.3 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 6 between pentazocine and naloxone

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pentazocine ($C_{19}H_{27}NO$) and naloxone ($C_{19}H_{21}NO_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pentazocine or naloxone from the *Sample solution*

r_S = peak response of pentazocine or naloxone from the *Standard solution*

C_S = concentration of the appropriate USP Pentazocine RS or USP Naloxone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pentazocine or naloxone in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• USP REFERENCE STANDARDS (11)

USP Naloxone RS

USP Pentazocine RS

Pentazocine Injection

DEFINITION

Pentazocine Injection is a sterile solution of Pentazocine in Water for Injection, prepared with the aid of Lactic Acid. It contains NLT 95.0% and NMT 105.0% of the labeled amount of pentazocine ($C_{19}H_{27}NO$).

IDENTIFICATION

• A.

Sample solution: Transfer a volume of Injection, equivalent to about 15 mg of lactic acid, to a 50-mL conical flask, add 1 mL of 2 N sulfuric acid, and mix. Add, dropwise, potassium permanganate solution (3.2 in 100) until a slight excess has been added, as evidenced by a violet color. [NOTE—The addition of a large excess of potassium permanganate may result in a false-negative test for lactate.]

Analysis: Moisten a piece of filter paper with a color-indicating solution (previously prepared by dissolving 250 mg of sodium nitroferrocyanide in water to make 9 mL of solution, adding 1 mL of morpholine, and mixing). Place the moistened filter paper over the conical flask opening, and heat the solution moderately.

Acceptance criteria: The acetaldehyde fumes produced turn the moistened filter paper blue.

• B. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181)

Standard solution: Dissolve 50 mg of USP Pentazocine RS in 25 mL of 0.01 N hydrochloric acid in a separator, and use this in place of the *Standard solution* specified in the chapter.

Sample solution: A volume of Injection equivalent to 50 mg of pentazocine

Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Diluent: Methanol and 6 N hydrochloric acid (500:500)

Column (ion-exchange): Place a pledget of glass wool in the base of a 6-mm (ID) tube equipped with a stopcock, and fill the tube to a height of about 25 mm with a styrene-divinylbenzene cation-exchange resin that has been previously soaked in 3 N hydrochloric acid for NLT 2 h. Pass 10 mL of methanol through the column, followed by 50 mL of 3 N hydrochloric acid, then wash the column with water until the eluate is neutral. [CAUTION—Do not permit the column to become dry at any time.]

Standard stock solution: 1.2 mg/mL of USP Pentazocine RS in *Diluent*

Standard solution: 0.12 mg/mL of USP Pentazocine RS in *Diluent* from *Standard stock solution*

Sample stock solution: Nominally equivalent to 6 mg/mL of pentazocine in water, diluted from an accurately measured volume of Injection.

Sample solution: Transfer 2.0 mL of *Sample stock solution* to the *Column*, then pass 100 mL of water through the *Column* at a rate of 2 mL/min, and discard the eluate. Place a 100-mL volumetric flask under the *Column*, and pass *Diluent* through the *Column* until approximately 95 mL of eluate has been collected. Remove the flask, and dilute with *Diluent* to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: 278 nm

Cell: 1 cm

Blank: *Diluent*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pentazocine ($C_{19}H_{27}NO$) in the portion of the Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Pentazocine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pentazocine in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

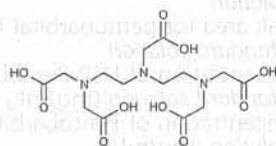
SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 5.8 USP Endotoxin Units/mg of pentazocine
- **PH (791):** 4.0–5.0
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS (11)**
USP Endotoxin RS
USP Pentazocine RS

Pentetic Acid



$C_{14}H_{23}N_3O_{10}$ 393.35

Glycine, *N,N*-bis[2-[bis(carboxymethyl)amino]ethyl].

Diethylenetriaminepentaacetic acid [67-43-6].

» Pentetic Acid contains not less than 98.0 percent and not more than 100.5 percent of $C_{14}H_{23}N_3O_{10}$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Pentetic Acid RS

Identification, Infrared Absorption (197K).

Residue on ignition (281): not more than 0.2%.

Delete the following:

• **Heavy metals, Method II (231):** 0.005%. • (Official 1-Jan-2018)

Limit of nitrilotriacetic acid—

Cupric acetate solution—Dissolve 20 g of cupric acetate in a mixture of 800 mL of water and 10 mL of glacial acetic acid. Adjust with 1 N sodium hydroxide to a pH of 4.2, dilute with water to obtain 1000 mL of solution, and filter.

Mobile phase—Prepare a mixture of 1600 mL of water, 40 mL of glacial acetic acid, 30.4 mL of 0.5 M dodecyltriethylammonium phosphate, and 20 mL of *Cupric acetate solution*. Adjust with 1 N sodium hydroxide to a pH of 4.0, dilute with water to obtain 2000 mL of solution, filter through a filter having a 0.5-μm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

Stock standard solution—Transfer about 50 mg of nitrilotriacetic acid, accurately weighed, to a 100-mL volumetric flask, dilute with *Cupric acetate solution* to volume, and mix.

Standard solution—Transfer 1.0 mL of the *Stock standard solution* to a 25-mL volumetric flask, dilute with *Cupric acetate solution* to volume, and mix. This solution contains about 0.02 mg of nitrilotriacetic acid per mL.

Test solution—Transfer about 2 g of Pentetic Acid, accurately weighed, to a 100-mL volumetric flask. Add about 70 mL of *Cupric acetate solution*, and swirl to dissolve. Sonicate, if necessary, to dissolve. Dilute with *Cupric acetate solution* to volume, and mix.

Resolution solution—Transfer 1.0 mL of the *Stock standard solution* to a 25-mL volumetric flask, dilute with *Test solution* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 290-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1 that has been highly deactivated (carbon loading of about 30%). The flow rate is about 1 mL per minute. Equilibrate the column by passing, in sequence, water, methanol, and water for about 15 minutes each, and then *Mobile phase* for about 45 minutes. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, R , between pentetic acid and nitrilotriacetic acid is not less than 2.0, and the relative retention times are about 0.6 for pentetic acid and 1.0 for nitrilotriacetic acid. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 20 μL) of the *Standard solution* and the *Test solution* into the chromatograph, and measure the responses for the major peaks. Calculate the percentage of nitrilotriacetic acid in the portion of Pentetic Acid taken by the formula:

$$10,000(C/W)(r_U/r_S)$$

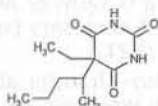
of which C is the concentration, in mg per mL, of nitrilotriacetic acid in the *Standard solution*, W is the weight, in mg, of Pentetic Acid taken to prepare the *Test solution*, and r_U and r_S are the nitrilotriacetic acid peak responses obtained from the *Test solution* and the *Standard solution*, respectively. The limit is 0.1%.

Iron—Using 1.5 g of specimen, proceed as directed in the test for *Iron* under *Edetic Acid*. The color of the test solution is not deeper than that of the solution containing the standard iron solution (0.01%).

Assay—Transfer about 200 mg of Pentetic Acid, accurately weighed, to a 125-mL conical flask, add 50 mL of water and 1.5 mL of 1 N sodium hydroxide, and swirl to dissolve the

specimen. Add 10 mL of 0.1 N ammonium thiocyanate, and mix. Add about 40 mL of methyl ethyl ketone, mix, and allow the layers to separate. Titrate with 0.05 N ferric ammonium sulfate VS, stirring continuously. As the titration proceeds, the aqueous phase turns from colorless to yellow, and the organic phase remains colorless. As the endpoint is approached, stop the titration, mix, and allow the layers to separate. Add 0.1-mL increments of 0.05 N ferric ammonium sulfate VS, mixing and allowing the layers to separate after each addition, until the organic layer turns from colorless to pink. Each mL of 0.05 N ferric ammonium sulfate consumed is equivalent to 19.668 mg of $C_{14}H_{23}N_3O_{10}$.

Pentobarbital



$C_{11}H_{18}N_2O_3$ 226.27
2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-methylbutyl)-, (±)-;
(±)-5-Ethyl-5-(1-methylbutyl)barbituric acid [76-74-4].

DEFINITION

Pentobarbital contains NLT 98.0% and NMT 102.0% of $C_{11}H_{18}N_2O_3$, calculated on the dried basis. Where the material is labeled as intended solely for veterinary use, Pentobarbital contains NLT 97.0% and NMT 102.0% of $C_{11}H_{18}N_2O_3$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197S)**
Sample solution: 7 in 100
Medium: Chloroform
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Mobile phase: 0.01 M monobasic potassium phosphate and acetonitrile (65:35). Adjust the pH to 3.5.
Standard solution: 0.1 mg/mL of USP Pentobarbital RS in *Mobile phase*

Sample stock solution: 1 mg/mL of Pentobarbital in *Mobile phase* (sonicate until dissolved)

Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection size: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 15,000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0% for pentobarbital

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{11}H_{18}N_2O_3$ in the portion of Pentobarbital taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak area from the *Sample solution*
- r_S = peak area from the *Standard solution*
- C_S = concentration of USP Pentobarbital RS in the *Standard solution* (mg/mL)
- C_U = concentration of Pentobarbital in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% of $C_{11}H_{18}N_2O_3$ on the dried basis; and 97.0%–102.0% of $C_{11}H_{18}N_2O_3$ on the dried basis, where the material is labeled as intended solely for veterinary use

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION (281):** NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-

Jan-2018)

Organic Impurities

• PROCEDURE

Mobile phase: Prepare as directed in the Assay.

Standard solution: 0.001 mg/mL of USP Pentobarbital RS in *Mobile phase*

Sample solution: 1 mg/mL of Pentobarbital in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection size: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 15,000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 15.0% for pentobarbital

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any impurity in the portion of Pentobarbital taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- r_U = peak area for any impurity from the *Sample solution*
- r_S = peak area for pentobarbital from the *Standard solution*
- C_S = concentration of USP Pentobarbital RS in the *Standard solution* (mg/mL)
- C_U = concentration of Pentobarbital in the *Sample solution* (mg/mL)
- F = relative response factor of the impurity (see *Impurity Table 1*)

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
6-Imino-5-ethyl-5-(1-methyl butyl) barbituric acid	0.39	1.5	0.2
5-Ethyl-5-(1-ethyl-propyl) barbituric acid ^a	0.93	1.0	0.1
Pentobarbital	1.0	—	—

^a Where the material is labeled as intended solely for veterinary use, the limit of 5-ethyl-5-(1-ethylpropyl) barbituric acid is 3.0%.

Impurity Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
5-Ethyl-5-(1,3-dimethylbutyl) barbituric acid	1.5	0.9	0.3
Unknown impurities	—	1.0	0.1
Total	—	—	0.5

* Where the material is labeled as intended solely for veterinary use, the limit of 5-ethyl-5-(1-ethylpropyl) barbituric acid is 3.0%.

SPECIFIC TESTS

- **Loss on drying** (731): Dry a sample at 105° for 2 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and storage:** Preserve in tight containers.
- **USP Reference Standards** (11)
USP Pentobarbital RS

Pentobarbital Sodium

$C_{11}H_{17}N_2NaO_3$ 248.25

2,4,6-(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-ethyl-5-(1-methylbutyl)-, monosodium salt.

Sodium 5-ethyl-5-(1-methylbutyl)barbiturate [57-33-0].

» Pentobarbital Sodium contains not less than 98.0 percent and not more than 102.0 percent of $C_{11}H_{17}N_2NaO_3$, calculated on the dried basis. Where the material is labeled as intended solely for veterinary use, Pentobarbital Sodium contains not less than 97.0 percent and not more than 102.0 percent of $C_{11}H_{17}N_2NaO_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Pentobarbital RS

Completeness of solution—Mix 1.0 g with 10 mL of carbon dioxide-free water: after 1 minute, the solution is clear and free from undissolved solid.

Identification—

A: Ultraviolet Absorption (197U)—

Solution: 10 µg per mL.

Medium: dilute ammonium hydroxide (1 in 200).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

C: Ignite about 200 mg: the residue effervesces with acids, and meets the requirements of the tests for *Sodium* (191).

pH (791): between 9.8 and 11.0, in the solution prepared in the test for *Completeness of solution*.

Loss on drying (731)—Dry it at 105° for 6 hours: it loses not more than 3.5% of its weight.

Delete the following:

• **Heavy metals, Method II** (231): 0.003%. • (Official 1-Jan-2018)

Related compounds—

Mobile phase—Prepare as directed in the *Assay*.

Standard solution—Dissolve an accurately weighed quantity of USP Pentobarbital RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.001 mg per mL.

Test solution—Transfer about 110 mg of Pentobarbital Sodium, accurately weighed, to a 100-mL volumetric flask, add about 80 mL of *Mobile phase*, and sonicate until dissolved. Dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the capacity factor, k' , is not less than 2.5; the column efficiency is not less than 15,000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 15.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of any impurity in the portion of Pentobarbital Sodium taken by the formula:

$$(248.25/226.27)(10,000/F)(C/W)(r_i/r_s)$$

in which 248.25 and 226.27 are the molecular weights of pentobarbital sodium and pentobarbital, respectively; F is the relative response factor of the impurity according to the table below; C is the concentration, in mg per mL, of USP Pentobarbital RS in the *Standard solution*; W is the weight, in mg, of Pentobarbital Sodium, on the dried basis, used to prepare the *Test solution*; r_i is the peak area for any impurity in the *Test solution*; and r_s is the peak area for pentobarbital in the *Standard solution*: the impurities meet the requirements given in the table below:

Compound Name	Relative Retention Time	Relative Response Factor	Limit (%)
6-Imino-5-ethyl-5-(1-methylbutyl)barbituric acid	about 0.39	1.5	0.2
5-Ethyl-5-(1-ethylpropyl)barbituric acid*	about 0.93	1.0	0.1
Pentobarbital	1.0	—	—
5-Ethyl-5-(1,3-dimethylbutyl)barbituric acid	about 1.5	0.9	0.3
Unknown impurities	—	1.0	0.1
Total	—	—	0.5

* Where the material is labeled as intended solely for veterinary use, the limit of 5-ethyl-5-(1-ethylpropyl) barbituric acid is 3.0%.

Assay—[NOTE—Use the value for *Loss on drying* obtained at the same time as the preparation of the *Test solution* in the test for *Related compounds* and the *Assay preparation* in the *Assay*.]

Mobile phase, *Standard preparation*, and *Chromatographic system*—Proceed as described in the *Assay* under *Pentobarbital*.

Assay preparation—Transfer about 110 mg of Pentobarbital Sodium, accurately weighed, to a 100-mL volumetric flask, add about 80 mL of *Mobile phase*, and sonicate until dissolved. Dilute with *Mobile phase* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{11}H_{17}N_2NaO_3$ in the portion of Pentobarbital Sodium taken by the formula:

$$(248.25/226.27)1000C(r_u / r_s)$$

in which 248.25 and 226.27 are the molecular weights of pentobarbital sodium and pentobarbital, respectively; C is the concentration, in mg per mL, of USP Pentobarbital RS in the *Standard preparation*; and r_u and r_s are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pentobarbital Sodium Injection

» Pentobarbital Sodium Injection is a sterile solution of Pentobarbital Sodium in a suitable solvent. Pentobarbital may be substituted for the equivalent amount of Pentobarbital Sodium, for adjustment of the pH. The Injection contains the equivalent of not less than 92.0 percent and not more than 108.0 percent of the labeled amount of $C_{11}H_{17}N_2NaO_3$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass. The Injection may be packaged in 50-mL containers.

Labeling—The label indicates that the Injection is not to be used if it contains a precipitate.

USP Reference standards (11)—

USP Endotoxin RS
USP Pentobarbital RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

Bacterial Endotoxins Test (85)—It contains not more than 0.8 USP Endotoxin Unit per mg of pentobarbital sodium.

pH (791): between 9.0 and 10.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Mobile phase—Prepare a filtered and degassed pH 3.5 mixture of 0.01 M monobasic potassium phosphate and acetonitrile (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Pentobarbital RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Quantitatively dilute a suitable volume of Injection with *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector, and a 4.6-mm \times 25-cm column that contains 5- μ m packing

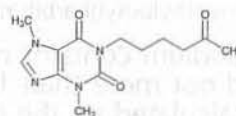
L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor, k' , is not less than 2.5; the column efficiency is not less than 15,000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_{11}H_{17}N_2NaO_3$ in the portion of Injection taken by the formula:

$$100(248.25/226.27)(C_s / C_u)(r_u / r_s)$$

in which 248.25 and 226.27 are the molecular weights of pentobarbital sodium and pentobarbital, respectively; C_s is the concentration, in mg per mL, of USP Pentobarbital RS in the *Standard preparation*; C_u is the final concentration, in mg per mL, of the *Assay preparation*; and r_u and r_s are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pentoxifylline



$C_{13}H_{18}N_4O_3$ 278.31
1*H*-Purine-2,6-dione, 3,7-dihydro-3,7-dimethyl-1-(5-oxohexyl)-;
1-(5-Oxohexyl)theobromine [6493-05-6].

DEFINITION

Pentoxifylline contains NLT 98.0% and NMT 102.0% of pentoxifylline ($C_{13}H_{18}N_4O_3$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: 1 g/L of perchloric acid

Mobile phase: Methanol, tetrahydrofuran, acetonitrile, and *Solution A* (2: 2.5: 15: 80)

System suitability solution: 0.024 mg/mL of caffeine and 0.048 mg/mL of USP Pentoxifylline RS in *Mobile phase*

Standard solution: 0.05 mg/mL of USP Pentoxifylline RS in *Mobile phase*

Sample solution: 0.05 mg/mL of Pentoxifylline in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 273 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 0.7 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 10.0 between caffeine and pentoxifylline, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pentoxifylline ($C_{13}H_{18}N_4O_3$) in the portion of Pentoxifylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response from the *Sample solution*
 r_S = peak response from the *Standard solution*
 C_S = concentration of USP Pentoxifylline RS in the *Standard solution* (mg/mL)
 C_U = concentration of Pentoxifylline in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **CHLORIDE AND SULFATE, Chloride** (221)

Sample: 2.0 g

Acceptance criteria: The *Sample* shows no more chloride than corresponds to 0.31 mL of 0.020 N hydrochloric acid (0.011%).

• **CHLORIDE AND SULFATE, Sulfate** (221)

Sample: 1.0 g

Acceptance criteria: The *Sample* shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.02%).

Delete the following:

• **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1, Jan-2018)

• **ORGANIC IMPURITIES**

Solution A and Mobile phase: Prepare as directed in the Assay.

System suitability solution: 0.7 µg/mL of caffeine and 350 µg/mL of USP Pentoxifylline RS in *Mobile phase*

Standard solution: 0.7 µg/mL of USP Pentoxifylline RS in *Mobile phase*

Sample solution: 350 µg/mL of Pentoxifylline in *Mobile phase*

Chromatographic system: Proceed as directed in the Assay except for the following:

Injection volume: 20 µL

Run time: NLT 5 times the retention time for pentoxifylline

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 10.0 between caffeine and pentoxifylline, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the areas of all the peaks in the *Sample solution*, except for that of pentoxifylline.

Calculate the percentage of each impurity in the portion of Pentoxifylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- r_U = peak response of each impurity from the *Sample solution*
 r_S = peak response of pentoxifylline from the *Standard solution*
 C_S = concentration of USP Pentoxifylline RS in the *Standard solution* (µg/mL)
 C_U = concentration of Pentoxifylline in the *Sample solution* (µg/mL)

Acceptance criteria

Individual impurities: NMT 0.2%

Total impurities: NMT 0.5%

SPECIFIC TESTS

• **COMPLETENESS OF SOLUTION** (641)

Sample solution: 1 g in 50 mL of carbon dioxide-free water

Acceptance criteria: Meets the requirements

• **ACIDITY**

Sample solution: 1 g in 50 mL of carbon dioxide-free water

Analysis: To the *Sample solution* add 1 drop of bromothymol blue TS.

Acceptance criteria: NMT 0.2 mL of 0.01 N sodium hydroxide is required to produce a color change.

• **LOSS ON DRYING** (731)

Analysis: Dry under vacuum at 60° for 3 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** (11)

USP Pentoxifylline RS

Pentoxifylline Compounded Oral Suspension

DEFINITION

Pentoxifylline Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of pentoxifylline ($C_{13}H_{18}N_4O_3$).

Prepare Pentoxifylline Compounded Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Pentoxifylline extended-release tablets* equivalent to	2 g of pentoxifylline
Purified Water, USP, a sufficient quantity to make	100 mL

*Trental 400-mg tablets, sanofi-aventis, Somerville, NJ.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Pentoxifylline extended-release tablets* in a suitable mortar, add *Purified Water* in small portions, and triturate to make a smooth paste. Add increasing volumes of *Purified Water* to make a pentoxifylline liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Purified Water* to bring to final volume, and mix well.

ASSAY

• **PROCEDURE**

Solution A: 50 mM monobasic potassium phosphate buffer, adjusted with phosphoric acid to a pH of 3.2

Mobile phase: Acetonitrile and *Solution A* (20:80). Pass through a filter of 0.45-µm pore size, and degas.

Internal standard solution: 100 µg/mL of caffeine in *Mobile phase*

Standard stock solution: 20 mg/mL of USP Pentoxifylline RS in *Mobile phase*

Standard solution: Pipet 1.0 mL of *Standard stock solution* into a 15-mL conical centrifuge tube, and add 9 mL of deionized water. Mix the sample for 30 s in a vortex mixer, and centrifuge for 30 min at 1250 × g. Pipet 50 µL of the supernatant into a separate borosilicate culture tube, dilute with 575 µL of *Mobile phase*, and add 625 µL of *Internal standard solution* to obtain a so-

lution having a nominal concentration of 80 µg/mL of pentoxifylline and 50 µg/mL of caffeine.

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of Oral Suspension into a 15-mL conical centrifuge tube, and add 9 mL of deionized water. Mix the sample for 30 s in a vortex mixer, and centrifuge for 30 min at 1250 × g. Pipet 50 µL of the supernatant into a separate borosilicate culture tube, dilute with 575 µL of *Mobile phase*, and add 625 µL of *Internal standard solution* to obtain a solution having a nominal concentration of 80 µg/mL of pentoxifylline and 50 µg/mL of caffeine.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for caffeine and pentoxifylline are about 0.42 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 10.0 between pentoxifylline and caffeine

Column efficiency: NLT 10,000 theoretical plates

Tailing factor: NMT 2.0 for the pentoxifylline peak

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pentoxifylline ($C_{13}H_{18}N_4O_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of pentoxifylline to the internal standard from the *Sample solution*

R_S = peak response ratio of pentoxifylline to the internal standard from the *Standard solution*

C_S = concentration of USP Pentoxifylline RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of pentoxifylline in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **PH (791):** 5.9–7.7

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored in a refrigerator or at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS (11)**
USP Pentoxifylline RS

Pentoxifylline Extended-Release Tablets

» Pentoxifylline Extended-Release Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of pentoxifylline ($C_{13}H_{18}N_4O_3$).

Packaging and storage—Preserve in well-closed containers. Protect from light, and store between 15° and 30°.

Labeling—The labeling indicates the *Dissolution Test* with which the product complies.

USP Reference standards (11)—

USP Pentoxifylline RS

Identification—

A: Infrared Absorption (197K)—

Test specimen—Finely powder not fewer than 5 Tablets. (A coarse screen may be used to separate the powder from the tablet film-coating if necessary.) Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of pentoxifylline, to a 15-mL centrifuge tube, add about 10 mL of methanol, cap the tube, and shake vigorously for about 5 minutes. Centrifuge for about 5 minutes to allow undissolved material to settle. Decant the supernatant into a suitable beaker, and evaporate the solution with the aid of a current of air to dryness at about 35°. Dissolve the residue in about 15 mL of methylene chloride, transfer to a separatory funnel, add about 10 mL of water, and shake. Allow the layers to separate, transfer the methylene chloride layer, and pass through a funnel partially filled with anhydrous sodium sulfate, collecting the filtrate in a small beaker. Evaporate the solution with the aid of a current of air to dryness at about 35°. Dissolve the residue so obtained in 8 to 10 mL of ether, and then chill in an ice bath, if necessary, to induce crystallization. Collect the crystals on filter paper, wash with about 2 mL of cold ether, and allow to air-dry. Prepare a mixture of about 1.5% (w/w) of the crystals in potassium bromide.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)—

TEST 1—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

Medium: water; 900 mL or 1000 mL.

Apparatus 2: 100 rpm.

Times: 1, 4, 8, and 12 hours.

Procedure—Determine the amount of $C_{13}H_{18}N_4O_3$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 274 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Pentoxifylline RS in the same *Medium*.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1	not more than 30%
4	between 30% and 55%
8	not less than 60%
12	not less than 80%

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: water; 900 mL.

Apparatus 2: 75 rpm.

Times: 1, 6, 10, and 20 hours.

Procedure—Proceed as directed for *Test 1*.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	between 8% and 30%
6	between 35% and 60%
10	between 53% and 78%
20	not less than 80%

TEST 3—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Times: 2, 8, 12, and 20 hours.

Procedure—Proceed as directed for *Test 1*.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
2	between 15% and 35%
8	between 55% and 75%
12	between 75% and 95%
20	not less than 85%

TEST 4—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Times: 1, 8, and 24 hours.

Procedure—Proceed as directed for *Test 1*.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	between 0% and 20%
8	between 35% and 60%
24	not less than 80%

TEST 5—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium: water; 900 mL.

Apparatus 2: 75 rpm.

Times: 1, 2, 4, 6, and 20 hours.

Procedure—Proceed as directed for *Test 1*, except to use the wavelength of maximum absorbance at about 264 nm instead of 274 nm.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	between 5% and 25%
2	between 10% and 35%
4	between 20% and 50%
6	between 30% and 60%
20	not less than 80%

TEST 6—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium: simulated gastric fluid (without enzymes); 900 mL.

Apparatus 2: 50 rpm.

Times: 2, 8, 12, and 24 hours.

Procedure—Proceed as directed for *Test 1*.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
2	between 10% and 30%
8	between 40% and 60%
12	between 55% and 75%
24	not less than 85%

TEST 7—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Times: 1, 3, 8, and 18 hours.

Procedure—Proceed as directed for *Test 1*.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	not more than 25%
3	between 25% and 45%
8	between 55% and 75%
18	not less than 80%

TEST 8—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

Medium: water; 900 mL.

Apparatus 2: 75 rpm.

Times: 1, 2, 4, 10, and 16 hours.

Procedure—Proceed as directed for *Test 1*.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	between 10% and 20%
2	between 15% and 35%
4	between 25% and 45%
10	between 55% and 75%
16	not less than 80%

TEST 9—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 9*.

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Times: 1, 3, 6, 12, and 18 hours.

Procedure—Proceed as directed for *Test 1*, except to use the wavelength of maximum absorbance at about 230 nm instead of 274 nm.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	between 0% and 20%
3	between 20% and 40%
6	between 30% and 60%
12	between 50% and 80%
18	not less than 80%

TEST 10—If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 10.

Medium: water; 900 mL.

Apparatus 2: 75 rpm.

Times: 1, 6, 12, and 20 hours.

Procedure—Proceed as directed for Test 1.

Tolerances—The percentages of the labeled amount of $C_{13}H_{18}N_4O_3$ dissolved at the times specified conform to the following table.

Time (hours)	Amount dissolved
1	not more than 20%
6	between 35% and 65%
12	between 60% and 90%
20	not less than 80%

Uniformity of dosage units (905): meet the requirements.

Chromatographic purity—

Perchloric acid solution, Mobile phase, Extracting solution, and System suitability solution—Prepare as directed in the Assay.

Standard solution—Dissolve an accurately weighed quantity of USP Pentoxifylline RS in Extracting solution containing an amount of methanol equal to 0.8% of the total volume to be used, and dilute quantitatively, and stepwise if necessary, with Extracting solution to obtain a solution having a known concentration of about 0.96 µg per mL.

Test solution—Transfer 10.0 mL of the first dilution filtrate from the Assay preparation to a 25-mL volumetric flask, dilute with Extracting solution to volume, and mix. The final concentration of pentoxifylline in this solution is about 0.32 mg per mL.

Chromatographic system (see Chromatography (621))—Proceed as directed in the Assay. Chromatograph the Standard solution, and record the peak responses for pentoxifylline as directed for Procedure: the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the Standard solution and the Test solution into the chromatograph, and allow the chromatogram to run five times longer than the retention time of the pentoxifylline peak. Record the chromatograms, and measure all the peak responses from the Test solution, except that for pentoxifylline. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$312C(r_i / r_s)$$

in which C is the concentration, in mg per mL, of USP Pentoxifylline RS in the Standard solution; r_i is the peak response for each impurity obtained from the Test solution; and r_s is the peak response for pentoxifylline obtained from the Standard solution: not more than 0.3% of any individual impurity is found; and not more than 1.0% of total impurities is found.

Assay—

Perchloric acid solution—Dissolve 1.0 g of perchloric acid in 1000 mL of water, and mix.

Mobile phase—Prepare a filtered and degassed mixture of Perchloric acid solution, acetonitrile, tetrahydrofuran, and methanol (80:15:2.5:2). Make adjustments if necessary (see System Suitability under Chromatography (621)).

Extracting solution—Prepare a mixture of water and alcohol (7:3).

System suitability solution—Transfer about 20 mg of USP Pentoxifylline RS and about 10 mg of caffeine, each accurately weighed, to a 25-mL volumetric flask. Add 0.2 mL of methanol, and swirl the flask to distribute the methanol.

Dilute with Extracting solution to volume, and mix. Pipet 3.0 mL of the resulting solution into a 50-mL volumetric flask, dilute with Extracting solution to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Pentoxifylline RS in Extracting solution containing an amount of methanol equal to 0.8% of the total volume to be used, and dilute quantitatively, and stepwise if necessary, with Extracting solution to obtain a solution having a known concentration of about 0.048 mg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 40 mg of pentoxifylline, to a 50-mL volumetric flask. Pipet 0.4 mL of methanol into the flask, and swirl for at least 1 minute. Add about 30 mL of Extracting solution, and sonicate for 60 minutes with occasional swirling of the flask. Add an additional 15 mL of Extracting solution, allow to cool to room temperature, dilute with Extracting solution to volume, and mix. Centrifuge or pass through a suitable filter. Reserve a portion of this first dilution for preparation of the Test solution in the Chromatographic purity test. Pipet 3.0 mL of the clear solution into a 50-mL volumetric flask, dilute with Extracting solution to volume, and mix.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 273-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 0.7 mL per minute. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure: the resolution, R , between caffeine and pentoxifylline is not less than 10.0. Chromatograph the Standard preparation, and record the peak responses for pentoxifylline as directed for Procedure: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of pentoxifylline ($C_{13}H_{18}N_4O_3$) in the portion of Tablets taken by the formula:

$$833C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Pentoxifylline RS in the Standard preparation; and r_u and r_s are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

Peppermint Spirit

DEFINITION

Peppermint Spirit contains, in each 100 mL, NLT 9.0 mL and NMT 11.0 mL of peppermint oil.

Peppermint Oil	100 mL
Peppermint, in coarse powder	10 g
Alcohol, a sufficient quantity to make	1000 mL

Macerate the peppermint leaves, freed as much as possible from stems and coarsely powdered, for 1 h in 500 mL of purified water, and then strongly express them. Add the moist, macerated leaves to 900 mL of alcohol, and allow the mixture to stand for 6 h with frequent agitation. Filter, and to the filtrate add the oil, and add alcohol to make the product measure 1000 mL.

ASSAY

• CONTENT OF PEPPERMINT OIL

Sample: 5.0 mL of Spirit

Analysis: Transfer the Sample to a Babcock bottle, graduated to 8%. Add 1.0 mL of kerosene, and mix. Add a

saturated calcium chloride solution, acidified with hydrochloric acid, almost to fill the bulb of the bottle. Rotate the bottle vigorously to ensure mixing, and then add a sufficient quantity of the calcium chloride solution to bring the separated oil into the neck of the bottle. Centrifuge at about 1500 rpm for 5 min, and read the volume of oil in the stem. Subtract five divisions for the kerosene added, and multiply the remaining number of divisions by 4.2 to obtain the volume, in mL, of peppermint oil in 100 mL of the Spirit.

Acceptance criteria: 9.0–11.0 mL

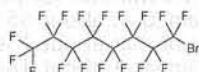
SPECIFIC TESTS

- **ALCOHOL DETERMINATION, Method II (611):** 79.0%–85.0% of C_2H_5OH

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.

Perflubron



C_8BrF_{17} 498.96

Octane, 1-bromo-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-heptafluoro-

1-Bromoheptafluorooctane.

Perfluorooctyl bromide [423-55-2].

» Perflubron contains not less than 98.0 and not more than 100.0 percent of C_8BrF_{17} .

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Perflubron RS

Identification—

A: Record the IR absorption spectrum, using a gas cell. The spectrum so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Perflubron RS.

B: The retention time of the major peak in the chromatogram of the test specimen, obtained as directed in the Assay, corresponds to that of USP Perflubron RS, similarly chromatographed.

Specific gravity (841): between 1.922 and 1.925.

Chromatographic purity—

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a split injection port with a split ratio, range of 1:45 to 1:100, a flame-ionization detector, and a 0.25-mm × 60-m column coated with a 1-μm film of phase G2. Hydrogen is used as the carrier gas. The chromatograph is programmed to maintain the column temperature at 35° for 7 minutes, then to increase the temperature at a rate of 20° per minute to 185°, and held at this temperature for 4.5 minutes. The injection port is maintained between 200° and 220° and the detector at a temperature above 200°.

Procedure—Inject a volume (about 0.2 μL) of Perflubron into the chromatograph, record the chromatogram, and measure the areas of the peak responses. Calculate the per-

centage of each individual impurity in the portion of Perflubron taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response of the individual impurity, and r_s is the sum of the responses of all the peaks: not more than 0.20% of any individual impurity is found.

Nonvolatile residue—Transfer 75 g of Perflubron to a tared evaporating dish, evaporate to dryness, and dry the residue at 105° for 1 hour: the weight of the residue so obtained does not exceed 1.5 mg (0.002%).

Assay—

Chromatographic system—Proceed as directed in the test for *Chromatographic purity*.

Procedure—Inject about 0.2 μL of Perflubron into the chromatograph, record the chromatograms, and measure the areas of the peak responses. Calculate the percentage of C_8BrF_{17} in the portion of Perflubron taken by the formula:

$$100(r_U / r_s)$$

in which r_U is the peak response for perflubron obtained from the test specimen, and r_s is the sum of the responses of all of the peaks.

Perflutren Protein-Type A Microspheres Injectable Suspension

» Perflutren Protein-Type A Microspheres Injectable Suspension is a sterile, nonpyrogenic suspension of microspheres produced by dispersing perflutren (octafluoropropane) gas in an aqueous solution of diluted sterile Albumin Human. It contains not less than 0.8 percent and not more than 1.2 percent protein. It may contain stabilizers, but contains no preservatives.

Packaging and storage—Preserve in single-dose, tight containers that contain perflutren gas in the headspace, and store in a refrigerator.

Labeling—Label it to indicate that perflutren gas is contained within the microspheres. The labeling also provides the following warnings: "Do not use if lower layer is cloudy or turbid, contains visible foreign matter, or if the contents do not appear as a homogeneous, opaque, milky-white suspension after mixing. Do not use if the upper white layer of product is absent. Do not inject air into the vial. Invert the vial, and gently rotate to resuspend the microspheres. Do not use if, after resuspension, the solution appears to be clear rather than opaque milky-white."

USP Reference standards (11)—
USP Endotoxin RS

Bacterial Endotoxins Test (85)—It contains not more than 0.5 USP Endotoxin Unit per mL of Perflutren Protein-Type A Microspheres Injectable Suspension.

Safety—It meets the requirements for biologics as set forth for *Safety Tests—Biologicals* under *Biological Reactivity Tests, In Vivo* (88).

Sterility Tests (71): meets the requirements.

pH (791): between 6.4 and 7.4.

Microsphere size and concentration—

Electrolyte solution—Use filtered and buffered saline electrolyte solution.¹

¹ Filtered and buffered saline electrolyte solution is available as ISOTON(®) II from Beckman Coulter, Inc., Fullerton, CA.

Diluent—Prepare a solution that contains, in each L of water, 1.5 g of sodium lauryl sulfate and 0.1 g of thimerosal. Prior to use, pass the solution through a 0.2- μ m nylon filter. [NOTE—The *Diluent* is to be used exclusively to prepare the *Reference stock solution* described below; it must not be used to prepare the *Test solution*.]

Reference stock solution—Transfer a quantity of NIST traceable microspheres suspension containing about 0.5 g of microspheres directly into a tared centrifuge tube equipped with a cap, and weigh.² Using the density and concentration of the microspheres obtained from the Certificate of Analysis, calculate the volume occupied by the microspheres and the number of microspheres in the portion taken. Calculate the target total volume, in mL, by dividing the number of microspheres in the portion taken by the target concentration of 2.0×10^8 microspheres per mL. Calculate the target *Diluent* volume by subtracting the volume occupied by the microspheres from the target total volume. Transfer the target volume of *Diluent* to the centrifuge tube containing the portion of microspheres taken, and mix the tube vigorously for 1 hour. The prepared *Reference stock solution*, which contains a suspension of microspheres with a target mean particle diameter of 5.2 μ m and a concentration of 2.0×10^8 microspheres per mL, is divided into smaller containers and stored at 5°.

Reference solution—Equilibrate the *Reference stock solution* to room temperature, and mix thoroughly. Immediately transfer 20 μ L of the *Reference stock solution* to a beaker containing 200 mL of *Electrolyte solution*, mix, and analyze immediately.

Blank solution—Use 200 mL of *Electrolyte solution*.

Test solution—Allow the Injectable Suspension to equilibrate to room temperature. Invert the vial, and gently rotate to resuspend the microspheres. [NOTE—After resuspension, the contents should appear as a homogeneous, opaque, milky-white suspension.] Immediately withdraw a 20- μ L aliquot, transfer to a beaker containing 200 mL of *Electrolyte solution*, mix, and analyze immediately.

Test apparatus—Use a multichannel particle analyzer that operates on the electrical zone-sensing principle.³ The analyzer is fitted and calibrated with an aperture tube having a 50- μ m orifice. The multichannel particle analyzer is equipped with software capable of data-smoothing, data extrapolation, distribution graphing, and data conversion. Analyze the *Blank solution*, the *Reference solution*, and the *Test solution* as directed for *Procedure*: the total count in the *Blank solution* is not more than 500; the mean particle diameter of microspheres in the *Reference solution* is within 5% of the mean particle diameter of microspheres in the *Reference stock solution*; the concentration of the *Reference solution* is within 10% of the concentration of the *Reference stock solution*; and the coincidence effect in the analysis of the *Test solution* is not more than 5%.

Procedure—Rinse the orifice of the aperture tube with *Electrolyte solution* before and after analyzing each preparation. Place the *Blank solution* in the apparatus, and adjust the vacuum on the sample stand so that the counting begins about 12 seconds after the analyzer is set to the counting position. Set the data acquisition to stop when one of the following conditions is met: preset length of time, preset volume, preset number of counts in any channel, or total counts. Collect the count versus the channel data for the *Blank solution*, and analyze using the data-smoothing, data extrapolation, distribution graphing, and data conversion features of the system software. In the same manner, analyze the *Reference solution* and the *Test solution*. The *Test solution* data are normalized and expressed as the number of microspheres per mL: the concentration of microspheres is between 5.0×10^8 and 8.0×10^8 per mL. Calculate the

percentage of microspheres less than 10 μ m in size in the portion of Injectable Suspension taken by the formula:

$$100(P_A / P_B)$$

in which P_A is the number of microspheres in the 1- to 10- μ m size range, and P_B is the number of microsphere particles in the 1- to 32- μ m size range. Not fewer than 93% of microsphere particles is smaller than 10 μ m.

Container headspace content—

Reference solutions—Use 99% perflutren reference standard⁴ (electronic grade perflutren gas of at least 99 molar % purity) and 60% perflutren reference standard⁴ (an electronic grade gas in air mixture containing 60 molar % perflutren gas).

Blank solution—Use ambient air.

Test solution—Use gas from the container headspace.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a thermal conductivity detector and a 0.53-mm \times 25-m fused-silica (porous layer open tubular) column coated with Al_2O_3 / KCl (aluminum oxide deactivated with potassium chloride).⁵ The carrier gas is helium with a flow rate adjusted to obtain a retention time of about 1.5 to 1.8 minutes for perflutren. The column temperature is maintained at about 65°, the injection port temperature is maintained at about 130°, and the detector temperature is maintained at about 180°. Chromatograph the *Reference solutions* as directed for *Procedure*: the resolution, R , between perflutren and air is not less than 2; and the relative standard deviation for replicate injections is not more than 5%. The measured value for the 99% perflutren reference standard is within 5% of the nominal value.

Procedure—Using a gas-tight syringe, separately inject 10 μ L of the *Blank solution*, the *Reference solutions*, and the *Test solution* into the chromatograph. Record the chromatograms, and measure the responses for the major peaks. The percentages of perflutren in the 99% perflutren reference standard and in the *Test solution* are calculated by comparing the peak areas in each with the peak areas obtained from the 60% perflutren reference standard. The container headspace of Injectable Suspension contains not less than 60% of perflutren gas.

Microsphere perflutren content—

Reference stock solutions—The 97% decafluorobutane reference standard is decafluorobutane gas of at least 97 molar % purity.⁶ The 5% decafluorobutane–5% perflutren reference standard is a mixture containing 5 molar % decafluorobutane gas and 5 molar % perflutren gas in air.⁷ The 99% perflutren reference standard is electronic grade perflutren gas of at least 99 molar % purity.⁴

Analysis vial—Transfer 100 μ L of 97% decafluorobutane reference standard gas and 100 μ L of glacial acetic acid to a 2-mL vial equipped with a septum cap.

Reference solution—Transfer 100 μ L of 99% perflutren reference standard and 0.75 mL of 1% Albumin Human to an *Analysis vial*, and incubate by mixing for at least 3 hours.

Test solution—Allow a vial of the Injectable Suspension to equilibrate to room temperature. Invert the vial, and gently rotate to resuspend the microspheres. [NOTE—After resuspension, the contents of the vial should appear as a homogeneous, opaque, milky-white suspension.] Withdraw 0.75 mL of Injectable Suspension, and transfer to another *Analysis vial*. Incubate the *Test solution* by mixing for at least 3 hours.

⁴ A suitable grade of perflutren (octafluoropropane) is available from Air Products and Chemicals, Inc., Allentown, PA.

⁵ The column is available from Varian U.S.A. Chrompack, Walnut Creek, CA, catalog number CP7517, as an Al_2O_3 /KCl PLOT column (0.53-mm ID, 25-m length).

⁶ The gas is available under product code 03047567SR-LD from Scott Medical Products, Plumsteadville, PA.

⁷ The mixture of the two gases in air is available under product code 03047566SR-LD from Scott Medical Products, Plumsteadville, PA.

² Microspheres with a mean particle diameter of 5 μ m are available as NIST traceable Dynospheres from Bangs Laboratories, Inc., Fishers, IN.

³ A suitable multichannel particle analyzer is available as the Multisizer Model IIe from Beckman Coulter, Inc., Fullerton, CA.

Chromatographic system (see *Chromatography* <621>).—Prepare as directed for *Container headspace content*. The carrier gas is helium with a flow rate adjusted to obtain the following retention times: 1.0 to 1.1 minutes for air, 1.3 to 1.5 minutes for perflutren, and 1.5 to 2.5 minutes for decafluorobutane. The column temperature is maintained at about 85°, and then after elution of the perflutren the temperature is increased at a rate of 50° per minute to 120°, and maintained at 120° for 2 minutes. The injection port temperature is maintained at about 130°, and the detector temperature is maintained at about 180°. Chromatograph the 5% decafluorobutane–5% perflutren reference standard and the *Reference solution* as directed for *Procedure*: the resolution, R , between air and perflutren is not less than 2; the resolution, R , between perflutren and decafluorobutane is not less than 5; the relative standard deviation determined from the perflutren peak response for the *Reference solution* is not more than 5%; and the relative standard deviation determined from the response ratios for replicate injections of the 5% decafluorobutane–5% perflutren reference standard is not more than 5%.

Procedure—Inject 20 μ L of the headspace gas from the vials containing the *Reference solution* and the *Test solution* into the gas chromatograph. Record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg per mL, of perflutren in the portion of *Injectable Suspension* taken by the formula:

$$(0.188M/V)(R_S / R_U)$$

in which M is the number of μ moles of decafluorobutane in an *Analysis vial* after addition of the *Injectable Suspension*; V is the volume, in mL, of *Injectable Suspension* added to the *Analysis vial*; and R_S and R_U are the peak area ratios of decafluorobutane to perflutren obtained from the *Reference solution* and the *Test solution*, respectively. The quantity of perflutren in the *Injectable Suspension* is between 0.11 mg per mL and 0.33 mg per mL.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products (Parenterals)*—*Product Quality Tests* <1>; with the exception of *Foreign and Particulate Matter*.

Assay for protein—

Diluted antifoam reagent—Transfer 100 μ L of antifoam reagent⁸ to a suitable container, and dilute with water to 10 mL.

Blank preparation—Transfer 500 μ L of Sodium Chloride Injection to a culture tube. Dilute the contents of the tube with water to 2 mL, and add 10 μ L of *Diluted antifoam reagent*.

Standard preparations—Transfer 25-, 50-, 62.5-, 75-, and 100- μ L aliquots of protein standard solution⁹ containing 8 g per dL into separate tubes. Dilute the contents of each tube with water to 2.00 mL, and add 10 μ L of the *Diluted antifoam reagent* to each tube. During the *Procedure*, the addition of 3.0 mL of biuret reagent TS to each of the tubes produces *Standard preparations* with protein concentrations of 0.4, 0.8, 1.0, 1.2, and 1.6 mg per mL.

Assay preparation—Equilibrate each container of *Injectable Suspension* to room temperature, and mix each for at least 5 minutes to ensure a homogeneous suspension. Vent the container, and transfer 500- μ L aliquots into separate tubes. Dilute the contents of each tube with water to 2 mL, and add 10 μ L of *Diluted antifoam reagent*.

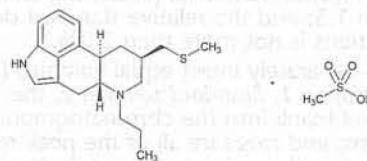
Procedure—To each of the tubes containing the *Blank preparation*, *Standard preparations*, and *Assay preparation*, add 3.0 mL of biuret reagent TS, mix, and allow to stand for 30 minutes, accurately timed, for maximum color development. The *Blank preparation*, *Standard preparations*, and *Assay preparation* are treated identically.

Using the *Blank preparation*, set the absorbance equal to zero. Determine the absorbance of each of the *Standard preparations* and the *Assay preparation* in 1-cm cells with a suitable spectrophotometer at a wavelength of 540 nm. Using linear regression, analyze the data obtained for each of the *Standard preparations*. Calculate the correlation coefficient, slope, and y-intercept values: the correlation coefficient is not less than 0.995. Calculate the quantity, in mg, of protein in each mL of the *Injectable Suspension* by the formula:

$$10[(A_U - y\text{-intercept})/\text{slope}]$$

in which 10 is the dilution factor; and A_U is the absorbance of the *Assay preparation*: the calculated quantity of protein in the *Injectable Suspension* is between 8 and 12 mg per mL.

Pergolide Mesylate



$C_{19}H_{26}N_2S \cdot CH_4O_3S$ 410.59

Ergoline, 8-[(methylthio)methyl]-6-propyl-, monomethanesulfonate, (8 β)-.

8 β -[(Methylthio)methyl]-6-propylergoline monomethanesulfonate [66104-23-2].

» Pergolide Mesylate contains not less than 97.5 percent and not more than 102.0 percent of $C_{19}H_{26}N_2S \cdot CH_4O_3S$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards <11>—

USP Pergolide Mesylate RS

USP Pergolide Sulfonate RS

(8 β)-8-[(Methylsulfinyl)methyl]-6-propyl-D-ergoline.

Identification, *Infrared Absorption* <197K>.

Specific rotation <781S>: between –17° and –23° at 20°.

Test solution: 10 mg per mL, in dimethylformamide.

Loss on drying <731>—Dry it in vacuum at 105° for 1 hour: it loses not more than 0.5% of its weight.

Residue on ignition <281>: not more than 0.1%.

Delete the following:

• **Heavy metals**, *Method II* <231>: 0.001%. • (Official 1-Jan-2018)

Chromatographic purity—

Solution A—Prepare a filtered and degassed mixture of 5.0 mL of morpholine with 995 mL of water, and adjust with phosphoric acid to a pH of 7.0.

Solution B—Prepare a filtered and degassed mixture of methanol, acetonitrile, and tetrahydrofuran (1:1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system* (see *System Suitability* under *Chromatography* <621>).

Standard solution 1—Dissolve an accurately weighed quantity of USP Pergolide Mesylate RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol

⁸ Available as Antifoam Reagent, catalog number 2210, from Dow Corning Corporation, Midland, MI.

⁹ Available as Bovine Serum Albumin, SRM 927c, Standard Reference Materials, National Institute of Standards and Technology, Gaithersburg, MD.

to obtain a solution having a known concentration of about 30 µg per mL.

Standard solution 2—Dilute 10.0 mL of *Standard solution 1* to 50 mL with methanol.

Test solution—Transfer about 60 mg of Pergolide Mesylate, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains base-deactivated packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	70	30	equilibration
0–35	70→0	30→100	linear gradient

Chromatograph *Standard solution 1*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 10,000 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of *Standard solution 1*, *Standard solution 2*, the *Test solution*, and a methanol blank into the chromatograph, record the chromatograms, and measure all of the peak responses. Disregard the contributions due to any peaks found in the methanol blank. The sum of the peak responses, excluding that of pergolide, from the *Test solution* is not more than the pergolide peak response obtained from *Standard solution 1* (0.5%), and no single peak response is more than the pergolide peak response obtained from *Standard solution 2* (0.1%).

Assay—**Diluent**—Dissolve 5 mg of methionine in 500 mL of 0.01 N hydrochloric acid. Add 500 mL of methanol, and mix.

Mobile phase—Prepare a solution of 0.009 M sodium 1-octanesulfonate containing 1.0 mL of glacial acetic acid per L. Prepare a filtered and degassed mixture of this solution, methanol, and acetonitrile (2:1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Resolution solution—Dissolve about 4 mg of USP Pergolide Sulfoxide RS and 8 mg of USP Pergolide Mesylate RS in 50 mL of *Diluent*.

Standard preparation—Dissolve an accurately weighed quantity of USP Pergolide Mesylate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.13 mg per mL.

Assay preparation—Transfer about 6.5 mg of Pergolide Mesylate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains base-deactivated packing L7. The flow rate is about 1 mL per minute. The column temperature is maintained at 40°. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between pergolide sulfoxide and pergolide is not less than 12.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pergolide peaks. Calculate the

quantity, in mg, of $C_{19}H_{26}N_2S \cdot CH_3O_3S$ in the portion of Pergolide Mesylate taken by the formula:

$$50C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Pergolide Mesylate RS in the *Standard preparation*; and *r_U* and *r_S* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pergolide Compounded Oral Suspension, Veterinary

DEFINITION

Pergolide Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of pergolide ($C_{19}H_{26}N_2S$).

Prepare Pergolide Compounded Oral Suspension, Veterinary, 1 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Pergolide (as Pergolide Mesylate, USP)	20 mg (26.11 mg)
Vehicle for Oral Suspension, NF	10 mL
Vehicle for Oral Solution, NF	10 mL

Connect an empty, calibrated, 35-mL Luer lock injection syringe to the port of a fluid-dispensing connector. Remove the plunger of another 35-mL Luer lock syringe, and set the plunger aside. Lock the barrel of this syringe onto the open port of the connector. Set this connected syringe apparatus in an upright, vertical position that is perpendicular to the work surface with the open syringe on top. Add the *Vehicle for Oral Suspension* and the *Pergolide Mesylate* into the open barrel. Replace the plunger on the open syringe, and invert the apparatus 180°. Apply 50 depressions to each syringe to mix. Consolidate the mixture into a single syringe. Disconnect the empty syringe. Add, via another 35-mL Luer lock injection syringe connected to the open port of the fluid-dispensing connector, a sufficient quantity of *Vehicle for Oral Solution* to bring the preparation to a final volume of 20 mL. Reattach the empty 35-mL syringe to the fluid-dispensing connector. Apply 50 depressions to each syringe to formulate a uniform suspension.

Alternatively, it may be prepared as follows. Add the *Pergolide Mesylate* to the mortar. Add the *Vehicle for Oral Suspension*, and mix to form a uniform paste. Add the *Vehicle for Oral Solution* in small portions almost to a final volume of 20 mL, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle for Oral Solution* to bring the preparation to a final volume of 20 mL, and mix well.

IDENTIFICATION

- A.** The retention time of the pergolide peak of the *Sample solution* corresponds to that of the *Standard solutions*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Solution A: 0.5 M sodium octanesulfonate solution and water (2:98), adjusted with glacial acetic acid to a pH of 2.2

Mobile phase: Acetonitrile and *Solution A* (50:50)
Diluent: Methanol and 0.01 N hydrochloric acid (50:50)

Standard stock solution: 1.0 mg/mL of USP Pergolide Mesylate RS in methanol prepared in low-actinic glassware

Standard solutions: Prepare five solutions of known concentrations of about 20, 10, 5, 2, and 1 µg/mL of pergolide mesylate by quantitatively diluting the *Standard stock solution* with *Mobile phase*. Use low-actinic glassware.

Sample solution: Transfer 500 µL of Oral Suspension, Veterinary to a 5-mL volumetric flask, and dilute with *Diluent* to volume. Further dilute an aliquot of the solution with *Mobile phase* to obtain a solution with a nominal concentration of 10 µg/mL of pergolide. Use low-actinic glassware.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 223 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Samples: *Standard solutions* and *Sample solution*

Suitability requirements

Tailing factor: NMT 2.0, *Standard solution* (20 µg/mL)

Relative standard deviation: NMT 2.0% for replicate injections, *Standard solution* (20 µg/mL)

Correlation coefficient: NLT 0.995, linear regression of the *Standard solutions*

Resolution: NLT 2.0, *Sample solution*

Analysis

Samples: *Standard solutions* and *Sample solution*

Generate a regression curve of peak height versus pergolide mesylate concentration, and calculate the equation for the linear regression line.

Calculate the percentage of the labeled amount of pergolide ($C_{19}H_{26}N_2S$) in the portion of Oral Suspension, Veterinary taken. Use the molecular weights of pergolide and pergolide mesylate, 314.50 and 410.60, respectively.

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **pH (791):** 4.0–4.2

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers, and store in a refrigerator.
- **BEYOND-USE DATE:** NMT 14 days after the date on which it was compounded when stored in a refrigerator
- **LABELING:** Label to state that it is to be well shaken before use, protected from light, and to state the *Beyond-Use Date*. Label to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS (11)**
USP Pergolide Mesylate RS

Pergolide Tablets

» Pergolide Tablets contain an amount of Pergolide Mesylate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of pergolide ($C_{19}H_{26}N_2S$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Pergolide Mesylate RS

USP Pergolide Sulfoxide RS

(8β)-8-[(Methylsulfinyl)methyl]-6-propyl-D-ergoline.

Thin-layer chromatographic identification test (201)—

Adsorbent: 0.25-mm layer of binder-free silica gel.

Test solution—Transfer a number of Tablets, equivalent to 1 mg of pergolide, to a separator containing 20 mL of methylene chloride and 10 mL of 0.1 N sodium hydroxide. Shake until the Tablets have disintegrated, allow the layers to separate, and drain the methylene chloride layer through a small funnel containing about 1 g of anhydrous sodium sulfate, collecting the filtrate in a suitable stoppered vessel. Wash the sodium sulfate with a few mL of methylene chloride, adding these washes to the filtrate, and evaporate to dryness under a stream of nitrogen. Redissolve the residue in 2 mL of a mixture of methylene chloride and methanol (1:1).

Standard solution: 0.65 mg per mL, in a mixture of methylene chloride and methanol (1:1).

Application volume: 20 µL.

Developing solvent system: a mixture of chloroform, methanol, and ethyl acetate (8:1:1). Allow the plate to equilibrate for about 10 minutes in the developing chamber prior to development.

Procedure—Proceed as directed in the chapter. Place the plate in a chamber containing iodine vapors, and locate the spots.

Dissolution (711)—

Medium: simulated gastric fluid TS (without enzymes) containing 20 µg of L-cysteine per mL; 500 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of $C_{19}H_{26}N_2S$ dissolved by employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, and triethylamine, (21:19:0.08). Adjust with phosphoric acid to a pH of 5.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Triethylamine phosphate suspension—Add 1.0 mL of triethylamine to 500 mL of acetonitrile, mix, and adjust with phosphoric acid to a pH of 5.0. A white precipitate will form. Stir continuously during use.

Resolution solution—Prepare a solution of USP Pergolide Mesylate RS and USP Pergolide Sulfoxide RS containing a known amount of each equivalent to the labeled amount of pergolide in each 500 mL of *Medium*.

Standard solution—Transfer about 16 mg of USP Pergolide Mesylate RS, accurately weighed, to a 250-mL volumetric flask, dissolve in 10.0 mL of methanol, dilute with *Medium* to volume, and mix. Dilute this solution quantitatively and stepwise with *Medium* to obtain a solution having a known concentration equivalent to the labeled amount of pergolide in each 500 mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a fluorometer set to an excitation wavelength of 224 nm and an emission wavelength of 350 nm and with a 4.6-mm × 15-cm column that contains base-deactivated packing L10. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between pergolide sulfoxide and pergolide is not less than 1.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections, determined from the pergolide peak, is not more than 2.0%.

Procedure—Immediately before injection, pipet 2.0 mL of *Triethylamine phosphate suspension*, continuously stirred, into a suitable container containing 5.0 mL of the solution for injection, and mix to obtain a clear solution. Separately inject equal volumes (about 200 µL) of the *Standard solution* and filtered portions of the solutions under test into the

chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the amount, in mg, of pergolide ($C_{19}H_{26}N_2S$) dissolved by the formula:

$$500C(314.50/410.60)(r_U / r_S)$$

in which C is the concentration, in μg per mL, of USP Pergolide Mesylate RS in the *Standard solution*; 314.50 and 410.60 are the molecular weights of pergolide and pergolide mesylate, respectively; and r_U and r_S are the peak areas obtained from the solution under test and the *Standard solution*, respectively.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{19}H_{26}N_2S$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Chromatographic purity—

Mobile phase, System suitability solution, Standard preparation, and Chromatographic system—Proceed as directed in the Assay.

Diluted standard preparation—Transfer 3.0 mL of the *Standard preparation* to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

Test preparation—Use the *Assay preparation*.

Procedure—Separately inject equal volumes (about 100 μL) of the *Diluted standard preparation* and the *Test preparation* into the chromatograph, and measure all of the peak responses. Calculate the percentage of each impurity in the Tablets by the formula:

$$20C(314.50/410.60)(r_i / r_S)$$

in which C is the concentration, in μg per mL, of USP Pergolide Mesylate RS in the *Diluted standard preparation*; 314.50 and 410.60 are the molecular weights of pergolide and pergolide mesylate, respectively; r_i is the peak response of the individual impurity obtained from the *Test preparation*; and r_S is the peak response of pergolide obtained from the *Diluted standard preparation*: not more than 6.0% of pergolide sulfoxide is found; not more than 0.5% of any individual impurity, excluding pergolide sulfoxide, is found; and not more than 1.0% of total impurities, excluding pergolide sulfoxide, is found.

Assay—

Mobile phase—Prepare a solution of 0.038 M sodium 1-octanesulfonate containing 0.0077 mg of methionine per mL and 2.45 mL of glacial acetic acid per L. Adjust with 5 N sodium hydroxide to a pH of 4.1. Prepare a filtered and degassed mixture of this solution and acetonitrile (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability solution—Prepare a solution of USP Pergolide Mesylate RS and USP Pergolide Sulfoxide RS in *Mobile phase* having a known concentration of about 6.5 μg per mL of pergolide mesylate and 0.1 μg per mL of pergolide sulfoxide.

Standard preparation—Dissolve an accurately weighed quantity of USP Pergolide Mesylate RS in *Mobile phase*, and dilute quantitatively and stepwise with *Mobile phase* to obtain a solution having a known concentration of about 6.5 μg per mL.

Assay preparation—Place 20 whole Tablets into a suitable stoppered container, add *Mobile phase*, shake and sonicate until the Tablets have dissolved, and quantitatively dilute to obtain a solution containing about 5 μg per mL of pergolide.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a fluorometer set to an excitation wavelength of 280 nm and an emission wavelength of 335 nm and with a 4.6-mm \times 7.5-cm col-

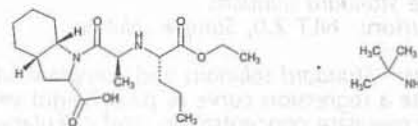
umn that contains base-deactivated packing L7. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 35°. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*; the resolution, R , between pergolide sulfoxide and pergolide is not less than 12.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 100 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and measure the responses for the major peaks. Calculate the quantity, in mg, of pergolide ($C_{19}H_{26}N_2S$) in the portion of Tablets taken by the formula:

$$0.001C(314.50/410.60)(r_U / r_S)$$

in which C is the concentration, in μg per mL, of USP Pergolide Mesylate RS in the *Standard preparation*; 314.50 and 410.60 are the molecular weights of pergolide and pergolide mesylate, respectively; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Perindopril Erbumine



$C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$ 441.6
1*H*-Indole-2-carboxylic acid, 1-[2-[[1-(ethoxycarbonyl)butylamino]-1-oxopropyl]octahydro-, [2*S*-[1[*R**(*R**)], 2 α , 3 $\alpha\beta$, 7 $\alpha\beta$]-, compound with 2-methyl-2-propanamine (1:1); (2*S*, 3 α *S*, 7 α *S*)-1-[(*S*)-*N*-[(*S*)-1-Carboxybutyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with *tert*-butylamine (1:1); (2*S*, 3 α *S*, 7 α *S*)-1-[(*S*)-2-[(*R*)-1-Ethoxy-1-oxopentan-2-ylamino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid [107133-36-8].

DEFINITION

Perindopril Erbumine contains NLT 98.0% and NMT 102.0% of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of *System suitability solution* 1, as obtained in the test for *Limit of Perindopril Related Compound I*.

ASSAY

• PROCEDURE

Buffer: Dissolve 0.92 g of sodium 1-heptanesulfonate in 1 L of water and add 1 mL of triethylamine. Adjust with a solution of perchloric acid and water (1:1) to a pH of 2.0.

Mobile phase: Acetonitrile and *Buffer* (35:65)

Standard solution: 0.1 mg/mL of USP Perindopril Erbumine RS in *Buffer*. Initially add *Buffer* to about 60% of the flask volume, sonicate to dissolve, and dilute with *Buffer* to volume.

Sample solution: 0.1 mg/mL of Perindopril Erbumine in *Buffer*. Initially add *Buffer* to about 60% of the flask volume, sonicate to dissolve, and dilute with *Buffer* to volume.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 215 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L7**Column temperature:** 60°**Flow rate:** 1 mL/min**Injection volume:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 0.5%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$) in the portion of Perindopril Erbumine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak area of perindopril from the *Sample solution* r_S = peak area of perindopril from the *Standard solution* C_S = concentration of USP Perindopril Erbumine RS in the *Standard solution* (mg/mL) C_U = concentration of Perindopril Erbumine in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

- **ORGANIC IMPURITIES**

Solution A: Proceed as directed for the *Buffer* as described in the *Assay*.**Solution B:** Acetonitrile**Mobile phase:** See *Table 1*.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
27	68	32
45	50	50
60	20	80
70	20	80
71	80	20
80	80	20

Diluent: *Solution B* and *Solution A* (20:80)**System suitability stock solution A:** 0.03 mg/mL of USP Imidazole RS in *Diluent***System suitability stock solution B:** 0.03 mg/mL each of USP Perindopril Erbumine RS, USP Perindopril Related Compound B RS, USP Perindopril Related Compound C RS, USP Perindopril Related Compound D RS, and USP Perindopril Related Compound F RS in *Diluent***System suitability solution:** Transfer 5 mL each of *System suitability stock solution A* and *System suitability stock solution B* to a 50-mL volumetric flask and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-μm pore size, discard the first 3 mL of filtrate, and use the clear filtrate.**Standard stock solution:** 0.03 mg/mL of USP Perindopril Erbumine RS in *Diluent* prepared as follows. Dissolve a suitable quantity of USP Perindopril Erbumine RS in 80% of the total volume of *Diluent*, sonicate for about 5 min, and dilute with *Diluent* to volume.**Standard solution:** 0.003 mg/mL of USP Perindopril Erbumine RS in *Diluent* from the *Standard stock solution*.

Pass through a suitable filter of 0.45-μm pore size and discard the first 3 mL of filtrate.

Sample solution: 3 mg/mL of Perindopril Erbumine in *Diluent* prepared as follows. Dissolve a suitable quantity of Perindopril Erbumine in 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-μm pore size and discard the first 3 mL of filtrate.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 210 nm**Column:** 4.0-mm × 25-cm; 4-μm packing L7**Column temperature:** 60°**Flow rate:** 1 mL/min**Injection volume:** 20 μL**System suitability****Sample:** *System suitability solution***Suitability requirements****Tailing factor:** NMT 1.5 for the perindopril peak**Relative standard deviation:** NMT 5.0% for the perindopril peak**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Perindopril Erbumine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_S = peak response of perindopril erbumine from the *Standard solution* C_S = concentration of USP Perindopril Erbumine RS in the *Standard solution* (mg/mL) C_U = concentration of Perindopril Erbumine in the *Sample solution* (mg/mL) F = relative response factor (see *Table 2*)**Acceptance criteria:** See *Table 2*. Disregard peaks less than 0.05%.**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Imidazole ^a	0.08	—	—
Perindopril related compound B ^b	0.42	1.20	0.3
Perindopril related compound C ^c	0.74	0.96	0.1
Perindopril related compound D ^d	0.85	0.98	0.1
Perindopril erbumine	1.0	—	—
Isopropyl perindopril ^e	1.22	1.00	0.40 (if present)

^a Imidazole is quantitated using the test for *Limit of Perindopril Related Compound A and Imidazole* and is included in the table for identification purposes only.^b (2S,3aS,7aS)-1-[(S)-2-[(S)-1-Carboxybutylamino]propanoyl]octahydro-1H-indole-2-carboxylic acid.^c (S)-2-[(3S,5aS,9aS,10aS)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1H)-yl]pentanoic acid.^d (S)-2-[(3S,5aS,9aS,10aR)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1H)-yl]pentanoic acid.^e (2S,3aS,7aS)-1-[(S)-2-[(S)-1-isopropoxy-1-oxopentan-2-ylamino]propanoyl]octahydro-1H-indole-2-carboxylic acid.^f (S)-Ethyl 2-[(3S,5aS,9aS,10aS)-3-methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1H)-yl]pentanoate.^g (2S,3aS,7aS)-1-[(2S)-2-[(5R)-3-cyclohexyl-2-(cyclohexylimino)-4-oxo-5-propylimidazolidin-1-yl]propanoyl]octahydro-1H-indole-2-carboxylic acid.^h Total impurities include all specified and unspecified impurities and imidazole from the test for *Limit of Perindopril Related Compound A and Imidazole*. Perindopril related compound A is not included.

Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Perindopril related compound F ^a	1.38	0.85	0.2
Perindopril imidazolidinone analogs	1.65	1.00	0.15 (if present)
Any individual unspecified impurity	—	—	0.10
Total impurities ^b	—	—	1

^a Imidazole is quantitated using the test for *Limit of Perindopril Related Compound A and Imidazole* and is included in the table for identification purposes only.

^b (2*S*,3*aS*,7*aS*)-1-[(*S*)-2-[(*S*)-1-Carboxybutylamino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid.

^c (*S*)-2-[(3*S*,5*aS*,9*aS*,10*aS*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid.

^d (*S*)-2-[(3*S*,5*aS*,9*aS*,10*aR*)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid.

^e (2*S*,3*aS*,7*aS*)-1-[(*S*)-2-[(*S*)-1-isopropoxy-1-oxopentan-2-ylamino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid.

^f (*S*)-Ethyl 2-[(3*S*,5*aS*,9*aS*,10*aS*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoate.

^g (2*S*,3*aS*,7*aS*)-1-[(*S*)-2-[(*S*)-3-cyclohexyl-2-(cyclohexylimino)-4-oxo-5-propylimidazolidin-1-yl]propanoyl]octahydro-1*H*-indole-2-carboxylic acid.

^h Total impurities include all specified and unspecified impurities and imidazole from the test for *Limit of Perindopril Related Compound A and Imidazole*. Perindopril related compound A is not included.

• LIMIT OF PERINDOPRIL RELATED COMPOUND A AND IMIDAZOLE

Solution A: Proceed as directed in *Organic Impurities*.

Solution B: Acetonitrile

Mobile phase: See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	83	17
5	83	17
6	20	80
20	20	80
21	83	17
33	83	17

Diluent: *Solution B* and *Solution A* (17:83)

Standard stock solution: 0.25 mg/mL of USP Perindopril Related Compound A RS and 0.1 mg/mL of USP Imidazole RS in *Diluent*. Sonicate if necessary.

Standard solution: 10 mg/mL of USP Perindopril Erbumine RS, 0.025 mg/mL of USP Perindopril Related Compound A RS, and 0.01 mg/mL of USP Imidazole RS in *Diluent* prepared as follows. Transfer a weighed amount of USP Perindopril Erbumine RS and a suitable amount of *Standard stock solution* to a suitable volumetric flask, and dissolve with sonication in *Diluent* equivalent to 60% of the final volume. Dilute with *Diluent* to volume, pass through a suitable filter of 0.45-μm pore size, and discard the first 3 mL of filtrate.

Sample solution: 10 mg/mL of Perindopril Erbumine in *Diluent*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detectors

Perindopril related compound A: UV 210 nm

Imidazole: UV 225 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Temperatures

Column: 60°

Sample cooler: 5°

Flow rate: 1 mL/min

Injection volume: 50 μL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for imidazole and perindopril related compound A are 1.0 and 1.4, respectively.]

Suitability requirements

Tailing factor: NMT 1.8 for perindopril related compound A

Relative standard deviation: NMT 5.0% for perindopril related compound A

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of perindopril related compound A or imidazole in the portion of Perindopril Erbumine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of perindopril related compound A or imidazole from the *Sample solution*

r_S = peak response of perindopril related compound A or imidazole from the *Standard solution*

C_S = concentration of USP Perindopril Related Compound A RS or USP Imidazole RS in the *Standard solution* (mg/mL)

C_U = concentration of Perindopril Erbumine in the *Sample solution* (mg/mL)

Acceptance criteria

Perindopril related compound A: NMT 0.25%

Imidazole: NMT 0.1%

• LIMIT OF PERINDOPRIL RELATED COMPOUND I

[NOTE—Perindopril related compound I is the epimer of perindopril: (2*S*,3*aS*,7*aS*)-1-[(*S*)-2-[(*R*)-1-ethoxy-1-oxopentan-2-ylamino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid.]

Solution A: Dissolve 5 g of potassium phosphate monobasic in 1900 mL of water. Adjust with triethylamine to a pH of 6.50 and add 100 mL of acetonitrile.

Solution B: Acetonitrile

Mobile phase: See Table 4.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	83	17
22	83	17
23	20	80
33	20	80
34	83	17
46	83	17

Diluent: *Solution B* and *Solution A* (17:83)

System suitability solution 1: 3 mg/mL of USP Perindopril Erbumine RS in *Diluent*. [NOTE—This solution is used in *Identification test B*.]

System suitability solution 2: 3 μg/mL of USP Perindopril Erbumine RS in *Diluent* from *System suitability solution 1*

Sample solution: 3 mg/mL of Perindopril Erbumine in *Diluent* prepared as follows. Dissolve a suitable quantity of Perindopril Erbumine in 80% of the total volume of

Diluent, sonicate for 5 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size and discard the first 3 mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *System suitability solution 2*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 5.0%

Analysis

Sample: *Sample solution*

Calculate the percentage of perindopril related compound I in the portion of Perindopril Erbumine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of perindopril related compound I from the *Sample solution*

r_T = total of all peak responses from the *Sample solution*

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

- **WATER DETERMINATION**, *Method 1a* (921): NMT 1.0%; 3.00%–4.50% for a monohydrate

- **OPTICAL ROTATION**, *Specific Rotation* (781S)

Sample solution: 10 mg/mL of Perindopril Erbumine in ethanol

Acceptance criteria: -66° to -69° , at 20°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers at controlled room temperature.

- **USP REFERENCE STANDARDS** (11)

USP Imidazole RS

USP Perindopril Erbumine RS

USP Perindopril Related Compound A RS

(2S,3aS,7aS)-Octahydro-1*H*-indole-2-carboxylic acid hydrochloride.

$C_{17}H_{28}N_2O_5 \cdot HCl$ 205.68

USP Perindopril Related Compound B RS

(2S,3aS,7aS)-1-[(S)-2-[(S)-1-Carboxybutylamino]propionyl]octahydro-1*H*-indole-2-carboxylic acid.

$C_{17}H_{28}N_2O_5$ 340.41

USP Perindopril Related Compound C RS

(S)-2-[(3S,5aS,9aS,10aS)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid.

$C_{17}H_{26}N_2O_4$ 322.40

USP Perindopril Related Compound D RS

(S)-2-[(3S,5aS,9aS,10aR)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid.

$C_{17}H_{26}N_2O_4$ 322.40

USP Perindopril Related Compound F RS

(S)-Ethyl 2-[(3S,5aS,9aS,10aS)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoate.

$C_{19}H_{30}N_2O_4$ 350.45

Perindopril Erbumine Tablets

DEFINITION

Perindopril Erbumine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$).

IDENTIFICATION

- **A.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the Assay.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Solution A: Dissolve 0.92 g of sodium 1-heptanesulfonate in 1 L of water and add 1 mL of triethylamine. Adjust with a solution of perchloric acid and water (1:1) to a pH of 2.0.

Mobile phase: Acetonitrile and *Solution A* (38:62)

Diluent: Acetonitrile and *Solution A* (40:60)

Standard solution: 0.08 mg/mL of USP Perindopril Erbumine RS in *Diluent* prepared as follows. Dissolve a suitable quantity of USP Perindopril Erbumine RS in 80% of the total volume of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size and discard the first 3 mL of filtrate.

Sample solution: Nominally equivalent to 0.08 mg/mL of perindopril erbumine in *Diluent* prepared as follows. Weigh and transfer the number of Tablets into a suitable volumetric flask, as indicated in *Table 1*.

Table 1

Tablet Strength (mg)	Number of Tablets (NLT)	Volumetric Flask (mL)
2	20	500
4	10	500
8	10	1000

Add *Diluent* to about 70% of the flask volume, shake mechanically for about 60 min at 180 rpm, and sonicate for 20 min. Dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- μ m pore size and discard the first 3 mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm \times 25-cm; 4- μ m packing L7

Temperatures

Column: 60°

Sample cooler: 5°

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: NLT 2.5 times the retention time of perindopril

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Perindopril Erbumine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of perindopril erbumine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Solution A: Proceed as directed in the Assay.

Mobile phase: Acetonitrile and Solution A (350:650)

Standard stock solution: 0.55 mg/mL of USP Perindopril Erbumine RS in acetonitrile

Standard solution: Prepare solutions of USP Perindopril Erbumine RS in Medium from the Standard stock solution, with final concentrations from Table 2.

Table 2

Tablet Strength (mg)	Concentration (mg/mL)
2	0.0022
4	0.0044
8	0.0088

Sample solution: Pass a portion of the solution under test through a suitable filter and discard the first 1 mL of filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 50°

Flow rate: 1.2 mL/min

Injection volume: 100 μL

Run time: NLT 1.6 times the retention time of perindopril

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$) dissolved.

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of the Standard solution (mg/mL)

L = label claim (mg/Tablet)

V = volume of Medium, 900 mL

Tolerances: NLT 85% (Q) of the labeled amount of perindopril erbumine ($C_{19}H_{32}N_2O_5 \cdot C_4H_{11}N$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Solution A: Proceed as directed in the Assay.

Solution B: Acetonitrile

Mobile phase: See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
27	68	32
45	50	50

Table 3 (Continued)

Time (min)	Solution A (%)	Solution B (%)
60	20	80
70	20	80
71	80	20
80	80	20

Diluent: Solution B and Solution A (20:80)

System suitability stock solution A: 0.03 mg/mL of USP Imidazole RS in Diluent

System suitability stock solution B: 0.12 mg/mL each of USP Perindopril Related Compound C RS and USP Perindopril Related Compound D RS in Diluent. Initially add 80% of the total volume of Diluent, sonicate for 5 min, and dilute with Diluent to volume.

System suitability solution: Accurately weigh about 1.5 mg each of USP Perindopril Related Compound B RS and USP Perindopril Related Compound F RS into a 50-mL volumetric flask. Add 30 mL of Diluent and sonicate for 5 min. Transfer 5.0 mL each of System suitability stock solution A, System suitability stock solution B, and Standard stock solution. Dilute with Diluent to volume.

Standard stock solution: 0.05 mg/mL of USP Perindopril Erbumine RS in Diluent prepared as follows. Dissolve a suitable quantity of USP Perindopril Erbumine RS in 80% of the total volume of Diluent, sonicate for 5 min, and dilute with Diluent to volume.

Standard solution: 0.005 mg/mL of USP Perindopril Erbumine RS in Diluent from the Standard stock solution. Pass through a suitable filter of 0.45-μm pore size and discard the first 3 mL of filtrate.

Sample solution: Nominally equivalent to 2 mg/mL of perindopril erbumine in Diluent prepared as follows.

Transfer a quantity equivalent to about 20 mg of perindopril erbumine from powdered Tablets (NLT 20) into a test tube. Pipet 10.0 mL of Diluent into the test tube, sonicate for about 10 min, and vortex for about 1 min. Pass through a suitable filter of 0.45-μm pore size and discard the first 3 mL of filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Proceed as directed in the Assay, except for the Run time. [NOTE—The run time is determined by the gradient from Table 3.]

System suitability

Sample: System suitability solution

Suitability requirements

Tailing factor: NMT 1.5 for the perindopril peak

Relative standard deviation: NMT 5.0% for the perindopril peak

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the Sample solution

r_S = peak response of perindopril erbumine from the Standard solution

C_S = concentration of USP Perindopril Erbumine RS in the Standard solution (mg/mL)

C_U = nominal concentration of perindopril erbumine in the Sample solution (mg/mL)

F = relative response factor for each individual impurity (see Table 4)

Acceptance criteria: See Table 4. Disregard peaks less than 0.1%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Imidazole ^a	0.08	—	—
Perindopril related compound B ^b	0.42	1.21	2.0
Perindopril related compound C ^c	0.74	0.97	0.5
Perindopril related compound D ^d	0.85	0.98	0.5
Perindopril erbumine	1.0	—	—
Perindopril related compound F ^e	1.38	0.86	3.0
Any unspecified impurity	—	—	0.2
Total impurities ^f	—	—	1.5

^a Imidazole is given for identification only and is not quantitated using this procedure.

^b (2S,3aS,7aS)-1-[(S)-2-[(S)-1-Carboxybutylamino]propanoyl]octahydro-1H-indole-2-carboxylic acid.

^c (S)-2-[(3S,5aS,9aS,10aS)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1H)-yl]pentanoic acid.

^d (S)-2-[(3S,5aS,9aS,10aR)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1H)-yl]pentanoic acid.

^e (S)-Ethyl 2-[(3S,5aS,9aS,10aS)-3-methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1H)-yl]pentanoate.

^f Total impurities excludes perindopril related compound F and perindopril related compound B.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in air-tight containers. Protect from heat and moisture.

• USP REFERENCE STANDARDS (11)

USP Imidazole RS

USP Perindopril Erbumine RS

USP Perindopril Related Compound B RS

(2S,3aS,7aS)-1-[(S)-2-[(S)-1-Carboxybutylamino]propanoyl]octahydro-1H-indole-2-carboxylic acid.

C₁₇H₂₈N₂O₅ 340.41

USP Perindopril Related Compound C RS

(S)-2-[(3S,5aS,9aS,10aS)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1H)-yl]pentanoic acid.

C₁₇H₂₆N₂O₄ 322.40

USP Perindopril Related Compound D RS

(S)-2-[(3S,5aS,9aS,10aR)-3-Methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1H)-yl]pentanoic acid.

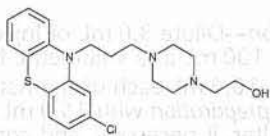
C₁₇H₂₆N₂O₄ 322.40

USP Perindopril Related Compound F RS

(S)-Ethyl 2-[(3S,5aS,9aS,10aS)-3-methyl-1,4-dioxodecahydropyrazino[1,2-a]indol-2(1H)-yl]pentanoate.

C₁₉H₃₀N₂O₄ 350.45

Perphenazine



C₂₁H₂₆ClN₃OS 403.97
Piperazineethanol, 4-[3-(2-chloro-10H-phenothiazin-10-yl)propyl];

4-[3-(2-Chlorophenothiazin-10-yl)propyl]-1-piperazineethanol [58-39-9].

DEFINITION

Perphenazine contains NLT 98.0% and NMT 102.0% of perphenazine (C₂₁H₂₆ClN₃OS), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**

- **B.** The retention time of the major peak of the *Diluted sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

ASSAY

• PROCEDURE

Sample solution: Dissolve 0.150 g of Perphenazine in 25 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis

Sample: *Sample solution*

Titrate the *Sample solution* with *Titrant*. Carry out a blank titration.

Calculate the percentage of perphenazine

(C₂₁H₂₆ClN₃OS) in the portion of Perphenazine taken:

$$\text{Result} = [(V - B) \times N \times F/W] \times 100$$

V = *Titrant* volume consumed by the *Sample* (mL)

B = *Titrant* volume consumed by the blank (mL)

N = normality of the *Titrant* (meq/mL)

F = equivalent weight of perphenazine, 202.0 mg/meq

W = *Sample* weight (mg)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

• ORGANIC IMPURITIES

Prepare the solutions immediately before use. Carry out the test protected from light.

Solution A: Acetonitrile and 7 g/L of monobasic sodium phosphate dihydrate in water (35:65)

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
10	80	20
33	30	70
48	100	0

System suitability solution: 2 mg/mL of USP Perphenazine RS, 0.002 mg/mL of USP Perphenazine Sulfoxide RS, and 0.002 mg/mL of USP Perphenazine Related Compound B RS in *Solution A*

Standard solution: 0.002 mg/mL of USP Perphenazine RS in *Solution A*

Sample solution: 2 mg/mL of Perphenazine in *Solution A*

Diluted sample solution: 0.002 mg/mL of Perphenazine in *Solution A* from the *Sample solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC
 Detector: UV 245 nm
 Column: 4.6-mm × 25-cm; 4-μm packing L7
 Column temperature: 30°
 Flow rate: 1.3 mL/min
 Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for relative retention times.]

Suitability requirements

Resolution: NLT 3.0 between perphenazine and perphenazine related compound B, *System suitability solution*

Tailing factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Diluted sample solution*. [NOTE—*Diluted sample solution* is used for *Identification test B*.]

Calculate the percentage of impurities in the portion of Perphenazine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response for each impurity from the *Sample solution*

r_S = peak response of perphenazine from the *Standard solution*

C_S = concentration of USP Perphenazine RS in the *Standard solution* (mg/mL)

C_U = concentration of Perphenazine in the *Sample solution* (mg/mL)

F = relative response factor (see Table 2)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Perphenazine sulfoxide	0.3	1.6	0.2
Perphenazine related compound B	0.8	1.0	0.5
Perphenazine	1.0	—	—
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry a sample in a vacuum at 65° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• USP REFERENCE STANDARDS (11)

USP Perphenazine RS

USP Perphenazine Related Compound B RS

2-[4-[3-(10*H*-Phenothiazin-10-yl)propyl]piperazin-1-yl]ethanol.

$C_{21}H_{27}N_3OS$ 369.52

USP Perphenazine Sulfoxide RS

2-Chloro-10-[3-[4-(2-hydroxyethyl)piperazin-1-yl]propyl]-10*H*-phenothiazine 5-oxide.

$C_{21}H_{26}ClN_3O_2S$ 419.97

Perphenazine Injection

» Perphenazine Injection is a sterile solution of Perphenazine in Water for Injection, prepared with the aid of Citric Acid. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{26}ClN_3OS$, as the citrate.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards (11)—

USP Endotoxin RS

USP Perphenazine RS

NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

Identification—Dilute 1 mL with methanol to 5 mL. Apply 5 μL each of this solution and a solution of USP Perphenazine RS in methanol containing 1 mg per mL to a suitable thin-layer chromatographic plate, coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram in a solvent system consisting of a mixture of acetone and ammonium hydroxide (200:1) until the solvent front has moved about 15 cm. Air-dry the plate, and spray lightly with a solution of iodoplatinic acid prepared by dissolving 100 mg of chloroplatinic acid in 1 mL of 1 N hydrochloric acid, adding 25 mL of potassium iodide solution (4 in 100), diluting with water to 100 mL, and adding 0.50 mL of formic acid: the R_f value of the principal spot obtained from the Injection corresponds to that obtained from the Standard solution.

Bacterial Endotoxins Test (85)—It contains not more than 35.7 USP Endotoxin Units per mg of perphenazine.

pH (791): between 4.2 and 5.6.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Acid-alcohol solution—Transfer 10 mL of hydrochloric acid to a 1000-mL flask containing 500 mL of alcohol and 300 mL of water. Dilute with water to volume.

Palladium chloride solution—Dissolve 100 mg of palladium chloride in a mixture of 1 mL of hydrochloric acid and 50 mL of water in a 100-mL volumetric flask, heating on a steam bath to effect solution. Cool, dilute with water to volume, and mix. Store in an amber bottle and use within 30 days. On the day of use, transfer 50 mL to a 500-mL volumetric flask, add 4 mL of hydrochloric acid and 4.1 g of anhydrous sodium acetate, dilute with water to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Perphenazine RS in *Acid-alcohol solution* to obtain a solution having a known concentration of about 150 μg per mL.

Assay preparation—Dilute 3.0 mL of Injection with *Acid-alcohol solution* to 100 mL in a volumetric flask.

Procedure—Mix 10.0 mL each of the *Assay preparation* and the *Standard preparation* with 15.0 mL of *Palladium chloride solution*, filter, if necessary, and concomitantly determine the absorbances of these solutions, against a reagent blank, in 1-cm cells at the wavelength of maximum absorbance at about 480 nm, with a suitable spectrophotometer.

Calculate the quantity, in mg, of $C_{21}H_{26}ClN_3OS$ in the volume of Injection taken by the formula:

$$0.1C(A_U / A_S)$$

in which C is the concentration, in μg per mL, of USP Perphenazine RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Perphenazine Oral Solution

» Perphenazine Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards (11)—
USP Perphenazine RS

NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

Identification—It meets the requirements for the *Identification* test under *Perphenazine Injection*.

Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Limit of perphenazine sulfoxide—

Mobile phase, *Resolution solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay*.

Test preparation—Transfer an accurately measured portion of Oral Solution, equivalent to about 16 mg of perphenazine, to a 200-mL volumetric flask, dissolve in and dilute with methanol to volume, mix, and filter.

Procedure—Inject a volume (about 10 μL) of the *Test preparation* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of perphenazine sulfoxide in the portion of Oral Solution taken by the formula:

$$100(r_1 / r_s)$$

in which r_1 is the peak response of perphenazine sulfoxide (relative retention time of about 0.72); and r_s is the sum of the responses of all the peaks: not more than 5.0% of perphenazine sulfoxide is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 0.01 M ammonium acetate, acetonitrile, and methanol (48:39:13). Adjust with glacial acetic acid to a pH of 4.5. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Resolution solution—Dissolve suitable quantities of brompheniramine maleate and USP Perphenazine RS in methanol to obtain a solution having known concentrations of about 40 μg per mL and 8 μg per mL, respectively.

Standard preparation—Dissolve an accurately weighed quantity of USP Perphenazine RS in methanol, dilute quantitatively, and stepwise if necessary, with methanol to obtain

a solution having a known concentration of about 8.0 μg per mL, and filter.

Assay preparation—Transfer an accurately measured portion of Oral Solution, equivalent to about 16 mg of perphenazine, to a 200-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a concentration of about 8.0 μg per mL, and filter.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm \times 30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for brompheniramine and 1.0 for perphenazine; and the resolution, R , between brompheniramine and perphenazine is not less than 3.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of perphenazine ($C_{21}H_{26}ClN_3OS$) in the portion of Oral Solution taken by the formula:

$$2000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Perphenazine RS in the *Standard preparation*; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Perphenazine Syrup

» Perphenazine Syrup contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards (11)—
USP Perphenazine RS

NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

Identification—Add 10 mL of water to a volume of Syrup, equivalent to about 4 mg of perphenazine, render alkaline by dropwise addition of sodium hydroxide to a pH of 11 to 12, and extract with four 5-mL portions of chloroform, combining the extracts through a bed of anhydrous sodium sulfate in a funnel into a beaker. Evaporate the extracts on a steam bath nearly to dryness, and dissolve the residue in 4 mL of methanol: the solution so obtained responds to the *Identification* test under *Perphenazine Injection*.

Uniformity of dosage units (905)—

FOR SYRUP PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume (698)—

FOR SYRUP PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Assay—

Acid-alcohol solution and Palladium chloride solution—Prepare as directed in the Assay under *Perphenazine Injection*.

Standard preparation—Dissolve an accurately weighed quantity of USP Perphenazine RS in *Acid-alcohol solution* to obtain a solution having a known concentration of about 160 µg per mL.

Assay preparation—Transfer an accurately measured volume of Syrup, equivalent to about 6 mg of perphenazine, to a 25-mL volumetric flask, dilute with water to volume, and mix. Transfer 10 mL to a 125-mL separator, add 25 mL of water, adjust with ammonium hydroxide to a pH of 10 to 11, and extract with four 20-mL portions of chloroform, filtering the extracts through anhydrous sodium sulfate. Evaporate the combined extracts on a steam bath with the aid of a stream of nitrogen to about 5 mL. Complete the evaporation without application of heat, and dissolve the residue in 15.0 mL of *Acid-alcohol solution*, filtering if necessary.

Procedure—Mix 10.0 mL each of the *Assay preparation* and the *Standard preparation* with 15.0 mL of *Palladium chloride solution*, filter if necessary, and concomitantly determine the absorbances of these solutions, against a reagent blank, in 1-cm cells at the wavelength of maximum absorbance at about 480 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of perphenazine ($C_{21}H_{26}ClN_3OS$) in each mL of the Syrup taken by the formula:

$$0.0375(C/V)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Perphenazine RS in the *Standard preparation*; V is the volume, in mL, of Syrup taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Perphenazine Tablets

» Perphenazine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{26}ClN_3OS$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—
USP Perphenazine RS

NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

Identification—Shake a portion of finely powdered Tablets, equivalent to about 5 mg of perphenazine, with about 10 mL of chloroform, filter, evaporate the filtrate on a steam bath nearly to dryness, and dissolve the residue in 5 mL of methanol: the solution so obtained responds to the *Identification test* under *Perphenazine Injection*.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{21}H_{26}ClN_3OS$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 257 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Perphenazine RS in the same medium.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{21}H_{26}ClN_3OS$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Acid-alcohol solution and Palladium chloride solution—Prepare as directed in the Assay under *Perphenazine Injection*.

Standard preparation—Prepare as directed in the Assay under *Perphenazine Syrup*.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer a portion of the powder, equivalent to about 4 mg of perphenazine, to a glass-stoppered conical flask, pipet into the flask 25 mL of *Acid-alcohol solution*, shake by mechanical means for 30 minutes, and centrifuge a portion of the mixture. The clear supernatant fluid is the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the Assay under *Perphenazine Injection*. Calculate the quantity, in mg, of $C_{21}H_{26}ClN_3OS$ in the portion of Tablets taken by the formula:

$$0.025C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Perphenazine RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Perphenazine and Amitriptyline Hydrochloride Tablets

» Perphenazine and Amitriptyline Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of perphenazine ($C_{21}H_{26}ClN_3OS$) and amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—
USP Amitriptyline Hydrochloride RS
USP Perphenazine RS

NOTE—Throughout the following procedures, protect test or assay specimens, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

Identification—Transfer a portion of powdered Tablets, equivalent to about 40 mg of perphenazine, to a 100-mL volumetric flask containing about 50 mL of alcohol. Agitate for 20 minutes, add alcohol to volume, mix, and filter or centrifuge. Separately prepare two Standard solutions containing 0.4 mg per mL of USP Perphenazine RS and USP Amitriptyline Hydrochloride RS, respectively, in alcohol. Separately apply 5 µL of the test solution and 5 µL of each Standard solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram using a solvent system consisting of a mixture of cyclohexane, ethyl acetate, and diethylamine (85:25:5) until the solvent front has moved about 15 cm. Remove the plate from the developing chamber, air-dry for 20 minutes, and examine the plate under short-wavelength UV light: the R_f values of the principal spots obtained from the test solution correspond to those obtained from the Standard solutions.

Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 60 minutes.

Procedure—[NOTE—Due to potential decrease in the recovery of perphenazine when multiple injections are made from a vial, no more than two withdrawals should be made from any single vial.] Determine the amounts of perphenazine and amitriptyline hydrochloride in solution in filtered portions of the solution under test, in comparison with a Standard solution having known concentrations of USP Perphenazine RS and USP Amitriptyline Hydrochloride RS in the same medium, as directed for *Procedure* in the *Assay*.

Tolerances—Not less than 75% (Q) of the labeled amounts of perphenazine ($C_{21}H_{26}ClN_3OS$) and amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$) is dissolved in 60 minutes.

Uniformity of dosage units (905): meet the requirements for *Content uniformity* with respect to perphenazine and to amitriptyline hydrochloride.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, methanol, and methanesulfonic acid (490:310:200:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Perphenazine RS in methanol, and dilute quantitatively with methanol to obtain a solution having a known concentration of about 0.8 mg per mL (*Solution P*). Transfer 4J mg of USP Amitriptyline Hydrochloride RS to a 50-mL volumetric flask, J being the ratio of the labeled amount, in mg, of amitriptyline hydrochloride to the labeled amount, in mg, of perphenazine per Tablet. Add 5.0 mL of *Solution P* and 20 mL of 0.2 N acetic acid, shake, and sonicate to dissolve the USP Reference Standards. Dilute with methanol to volume, and mix. Pipet 25 mL of this solution into a 100-mL volumetric flask, dilute with a mixture of methanol and 0.04 N acetic acid (3:2) to volume, and mix to obtain a *Standard preparation* having known concentrations of about 20 µg of USP Perphenazine RS per mL and about 20J µg of USP Amitriptyline Hydrochloride RS per mL.

Assay preparation—Transfer 10 Tablets to a 250-mL volumetric flask, add 100 mL of 0.2 N acetic acid, and shake the mixture until the Tablets have disintegrated. Add methanol to volume, mix, and filter. Dilute an accurately measured volume (V_F mL) of the clear filtrate quantitatively with a mixture of methanol and 0.04 N acetic acid (3:2) to obtain a solution (V_A mL) containing about 20 µg of perphenazine per mL, and filter through a membrane filter.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute, and is adjusted until the relative retention times for perphenazine and amitriptyline are about 1 and 1.5, respectively. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0% for replicate injections, and the resolution, R , between perphenazine and amitriptyline is not less than 4.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of perphenazine ($C_{21}H_{26}ClN_3OS$) in each Tablet taken by the formula:

$$0.25(C/10)(V_A/V_F)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP Perphenazine RS in the *Standard preparation*, V_A is the volume, in mL, of the *Assay preparation*, V_F is the volume, in mL, of the filtrate taken for the *Assay preparation*, and r_U and r_S are

the responses of the perphenazine peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of amitriptyline hydrochloride ($C_{20}H_{23}N \cdot HCl$) taken by the same formula, reading amitriptyline hydrochloride instead of perphenazine.

Pertussis Immune Globulin

» Pertussis Immune Globulin conforms to the regulations of the FDA concerning biologics (see *Biologics* (1041)). It is a sterile, nonpyrogenic solution of globulins derived from the blood plasma of adult human donors who have been immunized with pertussis vaccine such that each 1.25 mL contains not less than the amount of immune globulin to be equivalent to 25 mL of human hyperimmune serum. It may contain 0.3 M glycine as a stabilizing agent, and it contains a suitable preservative.

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 3 years after date of issue from manufacturer's cold storage (5°, 3 years).

Labeling—Label it to state that it is not intended for intravenous injection.

Petrolatum**DEFINITION**

Petrolatum is a purified mixture of semisolid hydrocarbons obtained from petroleum. It may contain a suitable stabilizer.

IMPURITIES**Inorganic Impurities**• **RESIDUE ON IGNITION** (281)

Sample: 2 g

Analysis: Heat the *Sample* in an open porcelain or platinum dish over a Bunsen flame.

Acceptance criteria: It volatilizes without emitting an acrid odor and yields NMT 0.1% of residue.

Organic Impurities• **PROCEDURE: ORGANIC ACIDS**

Sample solution: 20.0 g of Petrolatum in 100 mL of a 1 in 2 mixture of neutralized alcohol and water. Agitate thoroughly, and heat to boiling.

Analysis: Add 1 mL of phenolphthalein TS, and titrate rapidly with 0.1 N sodium hydroxide VS, with vigorous agitation to the production of a sharp pink endpoint, noting the color change in the alcohol-water layer.

Acceptance criteria: NMT 400 µL of 0.1 N sodium hydroxide is required.

SPECIFIC TESTS• **COLOR**

Standard solution: Ferric chloride CS and cobaltous chloride CS (3.8:1.2)

Sample: 10 g

Analysis: Melt the *Sample* on a steam bath, and pour 5 mL of the liquid into a clear-glass 15-mm × 150-mm test tube, keeping the petrolatum melted.

Acceptance criteria: The warm, melted liquid is not darker than 5 mL of the *Standard solution* in a similar tube; the comparison of the two being made in re-

flected light against a white background, and the petrolatum tube being held directly against the background at such an angle that there is no fluorescence.

- **SPECIFIC GRAVITY** (841): 0.815–0.880 at 60°
- **MELTING RANGE OR TEMPERATURE**, Class III (741): 38°–60°
- **CONSISTENCY**

Apparatus: A penetrometer fitted with a polished cone-shaped metal plunger weighing 150 g, having a detachable steel tip of the following dimensions: the tip of the cone has an angle of 30°, the point of the tip is truncated to a diameter of 0.381 ± 0.025 mm, the base of the tip is 8.38 ± 0.05 mm in diameter, and the length of the tip is 14.94 ± 0.05 mm.

The remaining portion of the cone has an angle of 90°, is 28 mm in height, and has a maximum diameter at the base of 65 mm. The containers for the test are flat-bottom metal cylinders that are 100 ± 6 mm in diameter and NLT 65 mm in height. They are constructed of at least 1.6-mm (16-gauge) metal, and are provided with well-fitting, water-tight covers.

Sample: Petrolatum

Analysis: Place the required number of containers in an oven, bring them and a quantity of the *Sample* to a temperature of $82 \pm 2.5^\circ$, and pour the *Sample* into one or more of the containers, filling to within 6 mm of the rim. Cool to $25 \pm 2.5^\circ$ over a period of NLT 16 h, protected from drafts. Two h before the test, place the containers in a water bath at $25 \pm 0.5^\circ$. If the room temperature is below 23.5° or above 26.5° , adjust the temperature of the cone to $25 \pm 0.5^\circ$ by placing it in the water bath.

Without disturbing the surface of the substance under test, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the test substance at a spot 25–38 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 s. Secure the plunger, and read the total penetration from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the test substance for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of the three or more readings, and conduct further trials to a total of 10 if the individual results differ from the average by more than $\pm 3\%$.

Acceptance criteria: The final average of the trials is NLT 10.0 mm and NMT 30.0 mm, indicating a consistency value of 100–300.

• ALKALINITY

Sample: 35 g

Analysis: Introduce the *Sample* into a suitable beaker, add 100 mL of boiling water, cover, and place on a stirring hot-plate maintained at the boiling point of water. After 5 min, allow the phases to separate. Draw off the separated water into a casserole, wash the petrolatum further with two 50-mL portions of boiling water, and add the washings to the casserole. To the pooled washings, add 1 drop of phenolphthalein TS, and boil.

Acceptance criteria: The solution does not acquire a pink color.

- **ACIDITY:** If the addition of phenolphthalein TS in the test for Alkalinity produces no pink color, add 0.1 mL of methyl orange TS.

Acceptance criteria: No red or pink color is produced.

• FIXED OILS, FATS, AND ROSIN

Sample: 10 g

Analysis: Digest the *Sample* with 50 mL of 5 N sodium hydroxide at 100° for 30 min. Separate the water layer, and acidify it with 5 N sulfuric acid.

Acceptance criteria: No oily or solid matter separates.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate the name and proportion of any added stabilizer.

Hydrophilic Petrolatum

DEFINITION

Prepare Hydrophilic Petrolatum as follows.

Cholesterol	30 g
Stearyl Alcohol	30 g
White Wax	80 g
White Petrolatum	860 g
To make	1000 g

Melt the *Stearyl Alcohol* and *White Wax* together on a steam bath, then add the *Cholesterol*, and stir until completely dissolved. Add the *White Petrolatum*, and mix. Remove from the bath, and stir until the mixture congeals.

White Petrolatum

DEFINITION

White Petrolatum is a purified mixture of semisolid hydrocarbons obtained from petroleum, and wholly or nearly decolorized. It may contain a suitable stabilizer.

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** (281)

Sample: 2 g

Analysis: Heat the *Sample* in an open porcelain or platinum dish over a flame.

Acceptance criteria: It volatilizes without emitting an acrid odor and yields NMT 0.05% of residue.

Organic Impurities

- **PROCEDURE: ORGANIC ACIDS**

Sample solution: 20.0 g of White Petrolatum in 100 mL of a 1 in 2 mixture of neutralized alcohol and water. Agitate thoroughly and heat to boiling.

Analysis: Add 1 mL of phenolphthalein TS, and titrate rapidly with 0.1 N sodium hydroxide VS, with vigorous agitation to the production of a sharp pink endpoint, noting the color change in the alcohol–water layer.

Acceptance criteria: NMT 400 μ L of 0.1 N sodium hydroxide is required.

SPECIFIC TESTS

• COLOR

Standard solution: Ferric chloride CS and water (1.6:3.4)

Sample: 10 g

Analysis: Melt the *Sample* on a steam bath, and pour 5 mL of the liquid into a clear-glass 16-mm \times 150-mm bacteriological test tube, keeping the petrolatum melted.

Acceptance criteria: The warm, melted liquid is not darker than 5 mL of the *Standard solution* in a similar tube; the comparison of the two being made in reflected light against a white background, and the petrolatum tube being held directly against the background at such an angle that there is no fluorescence.

- **SPECIFIC GRAVITY (841):** 0.815–0.880 at 60°
- **MELTING RANGE OR TEMPERATURE, Class III (741)** 38°–60°
- **CONSISTENCY**

Apparatus: A penetrometer fitted with a polished cone-shaped metal plunger weighing 150 g, having a detachable steel tip of the following dimensions: the tip of the cone has an angle of 30°, the point of the tip is truncated to a diameter of 0.381 ± 0.025 mm, the base of the tip is 8.38 ± 0.05 mm in diameter, and the length of the tip is 14.94 ± 0.05 mm.

The remaining portion of the cone has an angle of 90°, is 28 mm in height, and has a maximum diameter at the base of 65 mm. The containers for the test are flat-bottomed metal cylinders that are 100 ± 6 mm in diameter and NLT 65 mm in height. They are constructed of at least 1.6-mm (16-gauge) metal and are provided with well-fitting, water-tight covers.

Sample: White Petrolatum

Analysis: Place the required number of containers in an oven, and bring them and a quantity of Petrolatum to a temperature of $82 \pm 2.5^\circ$. Pour the Petrolatum into one or more of the containers, filling to within 6 mm of the rim. Cool to $25 \pm 2.5^\circ$ over a period of NLT 16 h, protected from drafts. Two h before the test, place the containers in a water bath at $25 \pm 0.5^\circ$. If the room temperature is below 23.5° or above 26.5° , adjust the temperature of the cone to $25 \pm 0.5^\circ$ by placing it in the water bath.

Without disturbing the surface of the substance under test, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the test substance at a spot 25–38 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 s. Secure the plunger, and read the total penetration from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the test substance for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of the three or more readings, and conduct further trials to a total of 10 if the individual results differ from the average by more than $\pm 3\%$.

Acceptance criteria: The final average of the trials is NLT 10.0 mm and NMT 30.0 mm, indicating a consistency value of 100–300.

- **ALKALINITY**

Sample: 35 g

Analysis: Introduce the *Sample* into a suitable beaker, add 100 mL of boiling water, cover, and place on a stirring hot-plate maintained at the boiling point of water. After 5 min, allow the phases to separate. Draw off the separated water into a casserole, wash the petrolatum further with two 50-mL portions of boiling water, and add the washings to the casserole. To the pooled washings, add 1 drop of phenolphthalein TS, and boil.

Acceptance criteria: The solution does not acquire a pink color.

- **ACIDITY**

Sample: Final solution of the test for *Alkalinity*, if the addition of phenolphthalein TS produced no pink color

Analysis: To the *Sample*, add 0.1 mL of methyl orange TS.

Acceptance criteria: No red or pink color is produced.

- **FIXED OILS, FATS, AND ROSIN**

Sample: 10 g

Analysis: Digest the *Sample* with 50 mL of 5 N sodium hydroxide at 100° for 30 min. Separate the water layer, and acidify it with 5 N sulfuric acid.

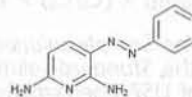
Acceptance criteria: No oily or solid matter separates.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **LABELING:** Label it to indicate the name and proportion of any added stabilizer.

Phenazopyridine Hydrochloride



$C_{11}H_{11}N_5 \cdot HCl$ 249.70
2,6-Pyridinediamine, 3-(phenylazo)-, monohydrochloride;
2,6-Diamino-3-(phenylazo)pyridine monohydrochloride
[136-40-3].

DEFINITION

Phenazopyridine Hydrochloride contains NLT 99.0% and NMT 101.0% of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

- **B. ULTRAVIOLET ABSORPTION (197U)**

Medium: Sulfuric acid in alcohol (1 in 360)

Sample solution: 5 µg/mL

Acceptance criteria: Meets the requirements

- **C.**

Standard solution: 0.02 mg/mL of USP Phenazopyridine Hydrochloride RS in the same medium as that in the *Sample solution*

Sample stock solution: 0.2 mg/mL of Phenazopyridine Hydrochloride in alcohol

Sample solution: Dilute the *Sample stock solution* with chloroform to obtain a solution with known concentration at 0.02 mg/mL.

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 µL

Developing solvent system: Chloroform, ethyl acetate, and methanol (85:10:5)

Spray reagent: 2 N hydrochloric acid

Analysis

Samples: *Standard solution* and *Sample solution*

Develop in *Developing solvent system*. Locate the spots by spraying the plate lightly with *Spray reagent*.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

- **PROCEDURE**

Standard solution: 5 µg/mL of USP Phenazopyridine Hydrochloride RS in the same medium as that in the *Sample solution*

Sample solution: Transfer about 100 mg of Phenazopyridine Hydrochloride to a 200-mL volumetric flask. Add about 100 mL of a mixture of sulfuric acid and alcohol (1 in 360), heat gently on a steam bath for 10 min, shake by mechanical means to dissolve, cool to room temperature, and dilute with alcoholic sulfuric acid to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with alcoholic sulfuric acid to volume, and mix. Transfer 5.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with alcoholic sulfuric acid to volume, and mix.

Instrumental conditions

Analytical wavelength: 390 nm (maximum absorbance)

Cell: 1 cm

Blank: Dilute alcoholic sulfuric acid (1 in 360)

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) in the portion of Phenazopyridine Hydrochloride taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Phenazopyridine Hydrochloride RS in the *Standard solution* ($\mu\text{g/mL}$)

C_U = concentration of the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

- **WATER-INSOLUBLE SUBSTANCES**

Sample: 2 g

Analysis: Dissolve the *Sample* in 200 mL of water, heat to boiling, then heat in a covered container on a steam bath for 1 h. Filter through a tared, fine-porosity, sintered-glass crucible, wash thoroughly with water, and dry at 105° to constant weight.

Acceptance criteria: The weight of the residue does not exceed 0.1% of the weight of Phenazopyridine Hydrochloride taken.

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1: Jan-2018)

- **ORDINARY IMPURITIES** (466)

Standard solutions: 0.04, 0.02, and 0.01 mg/mL of USP Phenazopyridine Hydrochloride RS in alcohol

Sample solution: 2.0 mg/mL in alcohol

Eluent: Chloroform, ethyl acetate, and methanol (85:10:5)

Visualization: Spray the plate with 5 N hydrochloric acid.

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **LOSS ON DRYING** (731)

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS** (11)

USP Phenazopyridine Hydrochloride RS

Phenazopyridine Hydrochloride Tablets

DEFINITION

Phenazopyridine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$).

IDENTIFICATION

- **A.**

Analysis: Transfer a quantity of finely ground Tablets, nominally equivalent to 50 mg of phenazopyridine hydrochloride, to a 125-mL separator, add 50 mL of water, 1 mL of 1 N hydrochloric acid, and 5 mL of a saturated sodium chloride solution, and shake to dissolve. Extract with two 25-mL portions of chloroform, and discard the chloroform. Add 5 mL of 1 N sodium

hydroxide to the aqueous solution, and extract with one 50-mL portion of chloroform. Transfer the chloroform layer to a second 125-mL separator, and wash with one 50-mL portion of 0.1 N sodium hydroxide. Filter the chloroform layer through a pledget of cotton previously washed with chloroform. Add 5 drops of hydrochloric acid to the filtrate, and evaporate on a steam bath under a current of air to dryness. Add 5 mL of alcohol, and evaporate. Dry the residue at 105° for 4 h.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the dried residue exhibits maxima only at the same wavelengths as that of a similar preparation of USP Phenazopyridine Hydrochloride RS.

ASSAY

- **PROCEDURE**

Buffer: 2.64 g of dibasic ammonium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 3.0, dilute with water to 1000 mL, and mix.

Mobile phase: Methanol and *Buffer* (500:500)

Standard solution: 0.5 mg/mL of USP Phenazopyridine Hydrochloride RS. Initially add 50% final volume of methanol, and swirl to dissolve. Dilute with *Buffer* to volume, and pass through a filter of 0.5- μm or finer pore size.

Sample solution: Transfer an amount nominally equivalent to 100 mg of phenazopyridine hydrochloride from finely powdered Tablets (NLT 20) to a 200-mL volumetric flask. Add 100 mL of methanol, and sonicate for 10 min. Add 75 mL of *Buffer*, and sonicate for an additional 10 min with occasional mixing. Dilute with *Buffer* to volume, and mix. Pass this solution through a filter of 0.5- μm or finer pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1400 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenazopyridine from the *Sample solution*

r_S = peak response of phenazopyridine from the *Standard solution*

C_S = concentration of USP Phenazopyridine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phenazopyridine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISSOLUTION** (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Detector: UV 422 nm

Standard solution: Known concentration of USP Phenazopyridine Hydrochloride RS in *Medium*

Sample solution: Filter portions of the solution under test, and suitably dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

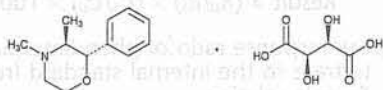
Tolerances: NLT 75% (Q) of the labeled amount of phenazopyridine hydrochloride ($C_{11}H_{11}N_5 \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Phenazopyridine Hydrochloride RS

Phendimetrazine Tartrate



$C_{12}H_{17}NO \cdot C_4H_6O_6$ 341.36
Morpholine, 3,4-dimethyl-2-phenyl-, (2*S*-*trans*)-, [*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1);
(2*S*,3*S*)-3,4-Dimethyl-2-phenylmorpholine L-(+)-tartrate (1:1) [50-58-8].

DEFINITION

Phendimetrazine Tartrate contains NLT 98.0% and NMT 102.0% of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B. ULTRAVIOLET ABSORPTION (197U)**
Sample solution: 1 mg/mL in methanol
Acceptance criteria: Meets the requirements
- **C. IDENTIFICATION TESTS—GENERAL, Tartrate (191)**

ASSAY

• PROCEDURE

Sample solution: Transfer an accurately weighed amount of 500 mg of Phendimetrazine Tartrate to a suitable beaker, and dissolve in 50 mL of glacial acetic acid.

Analysis: Add 1 drop of crystal violet TS to the *Sample solution*, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 34.14 mg of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$).

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%
- **CHLORIDE AND SULFATE, Chloride (221)**
Sample: 1.0 g
Acceptance criteria: 0.035%; the *Sample* shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid.
- **CHLORIDE AND SULFATE, Sulfate (221)**
Sample: 1.0 g
Acceptance criteria: 0.01%; the *Sample* shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid.

Delete the following:

- **HEAVY METALS (231):** NMT 10 ppm (Official 1-Jan-2018)

• ORGANIC IMPURITIES

Standard solution: An aqueous solution containing 100 mg/mL of USP Phendimetrazine Tartrate RS

Sample solution: 100 mg/mL of Phendimetrazine Tartrate in water

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: Acetone, methanol, and ammonium hydroxide (50:50:1)

Analysis

Develop the chromatogram in a suitable chamber with the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, view under short-wavelength UV light, and observe the location of the spots. Expose the plate to iodine vapors in a closed chamber.

Acceptance criteria: Yellow spots appear at the same locations as the spots observed under UV light, and the R_f value of the spot of the *Sample solution* corresponds to that of the *Standard solution*, and no other spot is obtained.

• L-Erythro ISOMER

Sample solution: Dissolve 3.0 g of Phendimetrazine Tartrate in 25 mL of sodium hydroxide solution (1 in 20) in a suitable separator. Add 25 mL of sodium hydroxide solution (1 in 2), swirl, and allow the phendimetrazine base to separate. Discard the lower, alkaline layer, and collect the upper layer, centrifuging, if necessary, to obtain a clear liquid.

Chromatographic system

Mode: GC

Detector: Flame ionization

Column: 25-m \times 0.25-mm capillary column, the inside wall of which is coated with a 0.4- μ m film of liquid phase G1

Carrier gas: Helium

Temperatures

Injection port: 250°

Column: 140°

Detector: 280°

Injection volume: 1.0 μ L

Injection type: Split ratio, 100:1

Analysis

Sample: *Sample solution*

[NOTE—The retention times for the *D-threo* isomer and the *L-erythro* isomer are about 8.5 and 9 min, respectively.]

Preferably using an electronic integrator, determine the areas of all peaks in the chromatogram.

Calculate the percentage of the *L-erythro* isomer in the *Sample solution* taken:

$$\text{Result} = (r_u/r_T) \times 100$$

r_u = peak area of the *L-erythro* isomer peak

r_T = sum of the areas of the *L-erythro* isomer peak and the *D-threo* isomer peak

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE (741):** 182°–188°, with decomposition, but the range between beginning and end of melting does not exceed 3°.

- **OPTICAL ROTATION, Specific Rotation (781S)**

Sample solution: 100 mg/mL in water

Acceptance criteria: +32° to +36°

- **PH (791):** 3.0–4.0, in a solution (1 in 40)

- **LOSS ON DRYING (731)**

Analysis: Dry to constant weight at 105°.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS (11)**

USP Phendimetrazine Tartrate RS

Phendimetrazine Tartrate Capsules

DEFINITION

Phendimetrazine Tartrate Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$).

IDENTIFICATION

- **A.**

Analysis: Shake a quantity of Capsule contents, nominally equivalent to 300 mg of phendimetrazine tartrate, with 50 mL of water. Filter, and transfer the filtrate to a 200-mL separator. Add 3 mL of 12.5 N sodium hydroxide, and extract with two 50-mL portions of chloroform. Extract the combined chloroform extracts in a 250-mL separator with two 15-mL portions of 0.5 N hydrochloric acid, and evaporate the combined aqueous extracts on a steam bath to dryness. Dissolve the residue in 5 mL of acetone, and add 50 mL of anhydrous ether to the solution. On standing, phendimetrazine hydrochloride crystallizes out. Filter the precipitate, wash with anhydrous ether, and dry at 105°.

Acceptance criteria: The phendimetrazine hydrochloride crystals so obtained melt at 189°–193°, but the range between the beginning and end of melting does not exceed 2°.

- **B. IDENTIFICATION TESTS—GENERAL, Tartrate (191)**

ASSAY

- **PROCEDURE**

Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 575 mL of water, add 400 mL of methanol and 25 mL of dilute acetic acid (14 in 100), and mix. Adjust with glacial acetic acid to a pH of 3.0 ± 0.1 , if necessary. Pass through a membrane filter of 0.45- μ m pore size, and degas.

Diluent: Dilute acetic acid (14 in 100), methanol, and water (2.5:40:57.5)

Internal standard solution: 0.1 mg/mL of salicylamide in Diluent

Standard solution: 0.7 mg/mL of USP Phendimetrazine Tartrate RS in Internal standard solution

Sample solution: Remove, as completely as possible, the contents of NLT 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to 35 mg of phendimetrazine tartrate, to a 50-mL volumetric flask. Add 25 mL of Internal standard solution, and sonicate for 15 min. Cool the solution to room temperature, dilute with Internal standard solution to volume, mix, and filter through a membrane filter of 0.45- μ m pore size.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 256 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: Standard solution

[NOTE—The relative retention times for salicylamide and phendimetrazine tartrate are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the analyte and internal standard peaks

Relative standard deviation: NMT 1.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of phendimetrazine tartrate to the internal standard from the Sample solution

R_S = peak response ratio of phendimetrazine tartrate to the internal standard from the Standard solution

C_S = concentration of USP Phendimetrazine Tartrate RS in the Standard solution (mg/mL)

C_U = nominal concentration of phendimetrazine tartrate in the Sample solution (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

- **DISSOLUTION (711)**

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 60 min

Solution A: 0.025 M monobasic potassium phosphate solution. Adjust with 1 N potassium hydroxide to a pH of 7.5.

Mobile phase: Acetonitrile and Solution A (65:35). Filter, and degas.

Standard solution: A known concentration of USP Phendimetrazine Tartrate RS, similarly prepared as the Sample solution

Sample solution: Filter a portion of the solution under test.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm \times 15-cm; packing L15

Flow rate: 1 mL/min

Injection volume: 50 μ L

System suitability

Sample: Standard solution

Suitability requirements

Relative standard deviation: NMT 3.0% from three replicate injections

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$) dissolved by comparison of the responses of the major peaks obtained from the Sample solution and the Standard solution.

Tolerances: NLT 70% (Q) of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Phendimetrazine Tartrate RS

Phendimetrazine Tartrate Tablets**DEFINITION**

Phendimetrazine Tartrate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$).

IDENTIFICATION• **A.**

Analysis: Shake a quantity of finely powdered Tablets, nominally equivalent to 300 mg of phendimetrazine tartrate, with 50 mL of water. Filter, and transfer the filtrate to a 200-mL separator. Add 3 mL of 12.5 N sodium hydroxide, and extract with two 50-mL portions of chloroform. Extract the combined chloroform extracts in a 250-mL separator with two 15-mL portions of 0.5 N hydrochloric acid, and evaporate the combined aqueous extracts on a steam bath to dryness. Dissolve the residue in 5 mL of acetone, and add 50 mL of anhydrous ether to the solution. On standing, phendimetrazine hydrochloride crystallizes out. Filter the precipitate, wash with anhydrous ether, and dry at 105°. **Acceptance criteria:** The phendimetrazine hydrochloride crystals so obtained melt at 189°–193°, but the range between the beginning and end of melting does not exceed 2°.

• **B. IDENTIFICATION TESTS—GENERAL, Tartrate (191)****ASSAY**• **PROCEDURE**

Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 575 mL of water, add 400 mL of methanol and 25 mL of dilute acetic acid (14 in 100), and mix. Adjust with glacial acetic acid to a pH of 3.0 ± 0.1 , if necessary. Pass through a membrane filter of 0.45- μ m pore size, and degas.

Diluent: Dilute acetic acid (14 in 100), methanol, and water (2.5: 40: 57.5)

Internal standard solution: 0.1 mg/mL of salicylamide in Diluent

Standard solution: 0.7 mg/mL of USP Phendimetrazine Tartrate RS in Internal standard solution

Sample solution: Transfer a portion of finely powdered Tablets from NLT 20 Tablets, nominally equivalent to 35 mg of phendimetrazine tartrate, to a 50-mL volumetric flask. Add 25 mL of Internal standard solution, and sonicate for 15 min. Cool the solution to room temperature, dilute with Internal standard solution to volume, mix, and pass through a membrane filter of 0.45- μ m pore size.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 256 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: Standard solution

[NOTE—The relative retention times for salicylamide and phendimetrazine tartrate are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the analyte and internal standard peaks

Relative standard deviation: NMT 1.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of phendimetrazine tartrate to the internal standard from the Sample solution

R_S = peak response ratio of phendimetrazine tartrate to the internal standard from the Standard solution

C_S = concentration of USP Phendimetrazine Tartrate RS in the Standard solution (mg/mL)

C_U = nominal concentration of phendimetrazine tartrate in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS• **DISSOLUTION (711)**

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 60 min

Solution A: 0.025 M monobasic potassium phosphate solution. Adjust with 1 N potassium hydroxide to a pH of 7.5.

Mobile phase: Acetonitrile and Solution A (65:35). Filter and degas.

Standard solution: USP Phendimetrazine Tartrate RS, similarly prepared as the Sample solution

Sample solution: Filter a portion of the solution under test.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 210 nm

Column: 4-mm \times 15-cm; packing L15

Flow rate: 1.0 mL/min

Injection volume: 50 μ L

System suitability

Sample: Standard solution

Suitability requirements

Relative standard deviation: NMT 3.0% from three replicate injections

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$) dissolved in comparison with the Standard solution.

Tolerances: NLT 70% (Q) of the labeled amount of phendimetrazine tartrate ($C_{12}H_{17}NO \cdot C_4H_6O_6$) is dissolved.

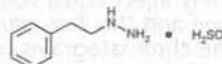
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**

USP Phendimetrazine Tartrate RS

Phenelzine Sulfate

$C_8H_{12}N_2 \cdot H_2SO_4$ 234.27

Hydrazine, (2-phenylethyl)-, sulfate (1:1).

Phenethylhydrazine sulfate (1:1). [156-51-4].

» Phenelzine Sulfate contains not less than 98.0 percent and not more than 102.0 percent of $C_8H_{12}N_2 \cdot H_2SO_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers, protected from heat and light.

USP Reference standards (11)—

USP Phenelzine Sulfate RS

Identification—

A: *Infrared Absorption* (197K).

B: Dissolve 100 mg in 5 mL of water, render the solution alkaline with 1 N sodium hydroxide, and add 1 mL of alkaline cupric tartrate TS: a red to yellow-red precipitate is formed.

C: A solution (1 in 10) meets the requirements of the tests for Sulfate (191).

Melting range (741): between 164° and 168°.

pH (791): between 1.4 and 1.9, in a solution (1 in 100).

Loss on drying (731)—Dry it at a pressure not exceeding 5 mm of mercury over silica gel at 80° for 2 hours: it loses not more than 1.0% of its weight.

Delete the following:

• **Heavy metals, Method I** (231): 0.002%. (Official 1-Jan-2018)

Limit of hydrazine—

Mobile phase—Prepare a filtered and degassed mixture of methanol and 1% monobasic ammonium phosphate solution (75:25). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard solution—Transfer an accurately weighed quantity of about 42.0 mg of hydrazine sulfate, equivalent to about 10 mg of hydrazine, to a 100-mL volumetric flask. Dissolve in water, sonicate for about 5 minutes, dilute with methanol to volume, and mix. Dilute an accurately measured volume of this solution quantitatively and stepwise with methanol to obtain a solution having a known concentration of 5.0 µg of hydrazine per mL. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, add 50 mL of methanol and 0.7 mL of ammonium hydroxide, and shake to mix. Add 0.5 mL of salicylaldehyde, shake by mechanical means for about 5 minutes, dilute with methanol to volume, sonicate for about 2 minutes, and mix.

Test solution—Transfer about 25.8 mg of Phenelzine Sulfate, accurately weighed, to a 200-mL volumetric flask, dissolve in about 50 mL of methanol, and sonicate. Add 0.7 mL of ammonium hydroxide, shake to mix, add 0.5 mL of salicylaldehyde, shake by mechanical means for about 5 minutes, dilute with methanol to volume, sonicate for about 2 minutes, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 340-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure*: the relative retention times for salicylaldehyde, phenelzine sulfate derivative, and hydrazine sulfate derivative are about 0.21, 0.47, and 1.0, respectively; the column efficiency determined from the analyte peak is not less than 4500 theoretical plates; the resolution, *R*, between phenelzine and salazine (derivatized hydrazine) is not less than 1.25; and the relative standard deviation for replicate injections is not more than 7%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas for salazine (derivatized hydrazine). Calculate the

percentage of hydrazine in the portion of Phenelzine Sulfate taken by the formula:

$$20(C/W)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of hydrazine base in the *Standard solution*; *W* is the weight, in mg, of Phenelzine Sulfate taken to prepare the *Test solution*; and *r_U* and *r_S* are the salazine peak areas obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.1% is found.

Ordinary impurities (466)—

Test solution: a mixture of methanol and water (1:1).

Standard solution: a mixture of methanol and water (1:1).

Eluant: acetone. [NOTE—Prewash the plate with *Eluant*, and dry.]

Visualization: 1.

Assay—

Ion-pair solution, Mobile phase, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay* under *Phenelzine Sulfate Tablets*.

Assay preparation—Transfer about 26 mg of Phenelzine Sulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of $C_8H_{12}N_2 \cdot H_2SO_4$ in the portion of Phenelzine Sulfate taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Phenelzine Sulfate RS in the *Standard preparation*; and *r_U* and *r_S* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenelzine Sulfate Tablets

» Phenelzine Sulfate Tablets contain an amount of phenelzine sulfate ($C_8H_{12}N_2 \cdot H_2SO_4$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of phenelzine ($C_8H_{12}N_2$).

Packaging and storage—Preserve in tight containers, protected from heat and light.

USP Reference standards (11)—

USP Phenelzine Sulfate RS

Identification—Extract a portion of powdered Tablets, equivalent to about 30 mg of phenelzine, with 10 mL of water, and filter: the filtrate responds to *Identification* tests B and C under *Phenelzine Sulfate*.

Disintegration (701)—Place 1 tablet in each of the 6 tubes of the basket and, if the tablets have a soluble external coating, immerse the basket in water at room temperature for 5 minutes. Then add a disk to each tube, and operate the apparatus, using simulated gastric fluid TS maintained at 37 ± 2° as the immersion fluid. After 30 minutes of operation in simulated gastric fluid TS, lift the basket from the fluid, and observe the tablets. If the tablets have not disintegrated completely, substitute simulated intestinal fluid TS maintained at 37 ± 2° as the immersion fluid. Continue the test for a total period of time, including previous exposure to water and simulated gastric fluid TS, of 1 hour and 30 minutes. Lift the basket from the fluid, and observe

the tablets: all of the tablets have disintegrated completely. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets: not less than 16 of the total 18 tablets tested disintegrate completely.

Uniformity of dosage units (905): meet the requirements.

Assay—

Ion-pair solution—Dissolve about 6.8 g of monobasic potassium phosphate and about 2.16 g of sodium 1-octanesulfonate in 1000 mL of water, and mix. Adjust with phosphoric acid to a pH of 3.0, and filter.

Mobile phase—Prepare a filtered and degassed mixture of *ion-pair solution* and methanol (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Phenelzine Sulfate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 258 µg per mL.

Assay preparation—Transfer not less than 20 Tablets to a suitable container, add about 300 mL of *Mobile phase*, and homogenize until dissolved. Transfer this solution to a 500-mL volumetric flask, dilute with *Mobile phase* to volume, mix, centrifuge, and filter, discarding the first 5 mL of the filtrate. Transfer a portion of the filtrate, equivalent to about 12.9 mg of phenelzine sulfate, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

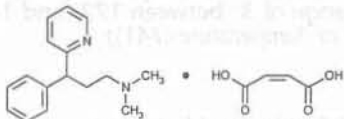
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 3.9-mm × 15-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of phenelzine (C₈H₁₂N₂) in the *Assay preparation* by the formula:

$$(136.20 / 234.27)(50C)(r_U / r_S)$$

in which 136.20 and 234.27 are the molecular weights of phenelzine and phenelzine sulfate, respectively, C is the concentration, in µg per mL, of USP Phenelzine Sulfate RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pheniramine Maleate



C₁₆H₂₀N₂ · C₄H₄O₄ 356.42

2-[α-[2-Dimethylaminoethyl]benzyl]pyridine bimalate.

N,N-Dimethyl-3-phenyl-3-(2-pyridyl)propylamine hydrogen maleate [132-20-7].

» Pheniramine Maleate contains not less than 98.0 percent and not more than 102.0 percent of C₁₆H₂₀N₂ · C₄H₄O₄, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Pheniramine Maleate RS

Identification, Infrared Absorption (197K).

Melting range, Class I (741): between 104° and 109°.

pH (791): between 4.5 and 5.5, in a solution (10 mg per mL).

Loss on drying (731)—Dry it in vacuum at 65° for 6 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.5%.

Delete the following:

• **Heavy metals, Method I** (231): 0.002%. • (Official 1-Jan-2018)

Chromatographic purity—

0.005 M Octane sulfonic acid—Transfer 1.08 g of sodium 1-octane sulfonate to a 1-liter volumetric flask. Dilute with 1.5% (v/v) acetic acid solution to volume, add 5.0 mL of triethylamine, mix, and filter.

Mobile phase—Prepare a filtered and degassed mixture of 0.005 M Octane sulfonic acid and acetonitrile (39:11). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability solution—Dissolve suitable quantities of phenylethyl alcohol and USP Pheniramine Maleate RS in water to obtain a solution containing about 3.6 and 0.24 mg per mL, respectively.

Test solution—Transfer about 24 mg of Pheniramine Maleate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 265-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 phenylethyl alcohol and 1.0 for pheniramine maleate, and the resolution, R , between phenylethyl alcohol and pheniramine maleate is not less than 2.0, the tailing factor is not more than 2.5, and the relative standard deviation for replicate injections is not more than 2.0%.

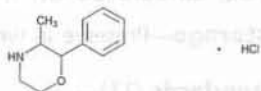
Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity (not including the solvent peak and maleic acid, if observed) in the portion of Pheniramine Maleate taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response for each impurity, and r_s is the sum of the responses of all of the peaks: not more than 0.5% of any individual impurity is found, and not more than 2.0% of total impurities is found.

Assay—Dissolve about 500 mg of Pheniramine Maleate, accurately weighed, in 25 mL of glacial acetic acid. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary corrections. Each mL of 0.1 N perchloric acid is equivalent to 17.82 mg of C₁₆H₂₀N₂ · C₄H₄O₄.

Phenmetrazine Hydrochloride



$C_{11}H_{15}NO \cdot HCl$ 213.70
Morpholine, 3-methyl-2-phenyl-, hydrochloride;
3-Methyl-2-phenylmorpholine hydrochloride [1707-14-8].

DEFINITION

Phenmetrazine Hydrochloride, dried at 105° for 2 h, contains NLT 98.0% and NMT 102.0% of phenmetrazine hydrochloride ($C_{11}H_{15}NO \cdot HCl$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197S)
Medium: Chloroform
Solution: 1 in 20
Acceptance criteria: Meets the requirements
- **B. ULTRAVIOLET ABSORPTION** (197U)
Medium: 0.5 N hydrochloric acid
Solution: 500 µg/mL
Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**
Diluent: 0.5 N hydrochloric acid
Standard solution: 0.5 mg/mL of USP Phenmetrazine Hydrochloride RS in Diluent
Sample solution: 0.5 mg/mL of previously dried Phenmetrazine Hydrochloride in Diluent

Instrumental conditions

Mode: UV
Analytical wavelength: Maximum absorbance at about 256 nm
Cell: 1 cm
Blank: 0.5 N hydrochloric acid

Analysis

Samples: Standard solution and Sample solution
Calculate the percentage of phenmetrazine hydrochloride ($C_{11}H_{15}NO \cdot HCl$) in the portion of Phenmetrazine Hydrochloride taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

- A_U = absorbance of the Sample solution
 A_S = absorbance of the Standard solution
 C_S = concentration of USP Phenmetrazine Hydrochloride RS in the Standard solution (mg/mL)
 C_U = concentration of Phenmetrazine Hydrochloride in the Sample solution (mg/mL)

Acceptance criteria: 98.0%–102.0% on sample dried at 105° for 2 h

OTHER COMPONENTS

• CONTENT OF CHLORIDE

Sample solution: Transfer about 350 mg, previously dried, to a 250-mL beaker. Add about 125 mL of water and 10 drops of sulfuric acid, and stir for 15 min with a magnetic stirrer.

Analysis: Titrate the solution potentiometrically with 0.1 N silver nitrate VS, using a silver–mercurous sulfate electrode system with a saturated salt bridge of potassium sulfate. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl.

Acceptance criteria: 16.3%–17.0%

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **CHLORIDE AND SULFATE, Sulfate** (221)
Sample: 2.0 g
Acceptance criteria: The Sample shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.01%).

Delete the following:

- **HEAVY METALS, Method II** (231): 0.001% (Official 1-Jan-2018)
- **ORDINARY IMPURITIES** (466)
Standard solution and Sample solution: Methanol
Eluant: A mixture of chloroform, absolute alcohol, and ammonium hydroxide (80:20:1)
Visualization: 1
Acceptance criteria: Meets the requirements

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE, Class Ia** (741): 172°–182°, but the range between beginning and end of melting does not exceed 3°.
- **PH** (791)
Sample solution: 1 in 40
Acceptance criteria: 4.5–5.5
- **LOSS ON DRYING** (731)
Analysis: Dry a sample at 105° for 2 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)
USP Phenmetrazine Hydrochloride RS

Phenmetrazine Hydrochloride Tablets

DEFINITION

Phenmetrazine Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of phenmetrazine hydrochloride ($C_{11}H_{15}NO \cdot HCl$).

IDENTIFICATION

- **A.**
Analysis: Dissolve 5 Tablets in 40 mL of water in a 250-mL separator. Add 3 mL of sodium hydroxide solution (1 in 2), and extract with two 50-mL portions of chloroform. Extract the combined chloroform extracts in a 250-mL separator with two 15-mL portions of 0.5 N hydrochloric acid, and evaporate the combined aqueous extracts on a steam bath to dryness. Dissolve the residue in 5 mL of acetone, and add 50 mL of anhydrous ether to the solution. On standing, phenmetrazine hydrochloride will crystallize out. Filter the precipitate, wash with anhydrous ether, and dry at 105°.
Acceptance criteria: The crystals so obtained melt within a range of 3° between 172° and 182° (see *Melting Range or Temperature* (741)).

ASSAY

• PROCEDURE

Diluent: 0.5 N hydrochloric acid
Standard solution: 500 µg/mL of USP Phenmetrazine Hydrochloride RS in Diluent
Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to 250 mg of phenmetrazine hydrochloride, to a 250-mL volumetric flask, add 125 mL of Diluent, shake by mechanical means for 1 h, and dilute with Diluent to volume. Transfer 50.0 mL of the solution to a 250-mL separator, add 5 mL of sodium hydroxide solu-

tion (1 in 2), and extract with four 50-mL portions of chloroform, collecting the chloroform extracts in a second 250-mL separator. Extract the combined chloroform extracts with six 15-mL portions of *Diluent*, collecting the aqueous extracts in a 100-mL volumetric flask, and dilute with *Diluent* to volume.

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 256 nm

Cell: 1 cm

Blank: 0.5 N hydrochloric acid

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenmetrazine hydrochloride ($C_{11}H_{15}NO \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Phenmetrazine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phenmetrazine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: USP Phenmetrazine Hydrochloride RS in *Medium*

Sample solution: A filtered portion of the solution under test, suitably diluted with *Medium*, if necessary, to a concentration similar to that of the *Standard solution* in the same *Medium*

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 256 nm

Analysis: Determine the percentage of the labeled amount of phenmetrazine hydrochloride ($C_{11}H_{15}NO \cdot HCl$) dissolved.

Tolerances: NLT 75% (Q) of the labeled amount of phenmetrazine hydrochloride ($C_{11}H_{15}NO \cdot HCl$) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

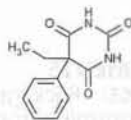
ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in tight containers.

• USP REFERENCE STANDARDS (11)

USP Phenmetrazine Hydrochloride RS

Phenobarbital



$C_{12}H_{12}N_2O_3$ 232.24

2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-phenyl-
5-Ethyl-5-phenylbarbituric acid [50-06-6].

» Phenobarbital contains not less than 98.0 percent and not more than 101.0 percent of $C_{12}H_{12}N_2O_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Phenobarbital RS

Identification—

A: The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Phenobarbital RS. If a difference appears, dissolve portions of both the test specimen and the USP Reference Standard in a suitable solvent, evaporate the solutions to dryness, and repeat the test on the residues.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

Melting range (741): between 174° and 178°, but the range between beginning and end of melting does not exceed 2°.

Loss on drying (731)—Dry it at 105° for 2 hours: it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.15%.

Assay—

pH 4.5 Buffer solution—Dissolve about 6.6 g of sodium acetate trihydrate and 3.0 mL of glacial acetic acid in 1000 mL of water, and adjust, if necessary, with glacial acetic acid to a pH of 4.5 ± 0.1.

Mobile phase—Prepare a filtered and degassed mixture of *pH 4.5 Buffer solution* and methanol (3:2), making adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard solution—Dissolve a sufficient quantity of caffeine in a mixture of methanol and *pH 4.5 Buffer solution* (1:1) to obtain a solution having a concentration of about 125 µg per mL.

Standard preparation—Dissolve about 20 mg of USP Phenobarbital RS, accurately weighed, in 15.0 mL of *Internal standard solution*. Sonicate if necessary.

Assay preparation—Transfer about 20 mg of Phenobarbital, accurately weighed, to a conical flask, add 15.0 mL of *Internal standard solution*, mix, and sonicate for 15 minutes. Filter through a membrane filter (0.5 µm or finer porosity) before use.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R , between the analyte and the internal standard peaks is not less than 1.2, the tailing factor for the analyte and the internal standard peaks is not greater than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for caffeine and 1.0 for phenobarbital. Calculate the quantity, in mg, of $C_{12}H_{12}N_2O_3$ in the portion of Phenobarbital taken by the formula:

$$W(R_U/R_S)$$

in which W is the weight, in mg, of USP Phenobarbital RS taken for the *Standard preparation*, and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenobarbital Oral Solution

» Phenobarbital Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of phenobarbital ($C_{12}H_{12}N_2O_3$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Phenobarbital RS

Identification—

A: Place 10 mL of Oral Solution in a separator containing 20 mL of water, add 5 mL of 1 N sodium hydroxide, and extract with two 10-mL portions of chloroform, discarding the chloroform extracts. Add 5 mL of 3 N hydrochloric acid, and extract with two 25-mL portions of chloroform, filtering the extracts through paper into a beaker. Remove the chloroform by evaporation on a steam bath, and dry the residue at 105° for 2 hours: the residue so obtained meets the requirements for Identification test A under Phenobarbital.

B: The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, both relative to the internal standard, as obtained in the Assay.

Alcohol Determination, Method II (611): between 12.0% and 15.0% of C_2H_5OH .

Assay—

pH 4.5 Buffer solution, Mobile phase, and Chromatographic system—Prepare as directed in the Assay under Phenobarbital.

Diluent—Prepare a mixture of methanol and pH 4.5 Buffer solution (2:1).

Internal standard solution—Dissolve a sufficient quantity of caffeine in Diluent to obtain a solution having a concentration of about 1.7 mg per mL.

Standard preparation—Transfer about 33 mg of USP Phenobarbital RS, accurately weighed, to a 25-mL volumetric flask containing 2.0 mL of Internal standard solution. Dilute with Diluent to volume, and mix.

Assay preparation—Transfer a quantity of Oral Solution, equivalent to about 33 mg of phenobarbital, to a 25-mL volumetric flask containing 2.0 mL of Internal standard solution. Dilute with Diluent to volume, and mix.

Procedure—Proceed as directed for Procedure in the Assay under Phenobarbital. Calculate the quantity, in mg, of phenobarbital ($C_{12}H_{12}N_2O_3$) in the portion of the Oral Solution taken by the formula:

$$W(R_U / R_S)$$

in which the terms are as defined therein.

Phenobarbital Compounded Oral Suspension

DEFINITION

Phenobarbital Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of phenobarbital ($C_{12}H_{12}N_2O_3$).

Prepare Phenobarbital Compounded Oral Suspension 10 mg/mL as follows (see Pharmaceutical Compounding—Nonsterile Preparations (795)).

Phenobarbital tablets ^a equivalent to	1.2 g of phenobarbital
Vehicle: a 1:1 mixture of Ora-Sweet ^b (regular or sugar-free) and Ora-Plus, ^b a sufficient quantity to make	120 mL

^aPhenobarbital 60-mg tablets, Excellium Pharmaceutical, Inc., Fairfield, NJ.

^bPaddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of Phenobarbital tablets in a suitable mortar, and comminute to a fine powder with a pestle. Add the Vehicle in small portions, and triturate to make a smooth paste. Add increasing volumes of the Vehicle to make a phenobarbital liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the Vehicle to bring to final volume, and mix well.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile and water (30:70). Adjust with dilute sulfuric acid to a pH of 3.0.

Standard stock solution: 0.4 mg/mL of USP Phenobarbital RS in Mobile phase

Standard solution: 20 µg/mL of phenobarbital prepared from Standard stock solution and Mobile phase

Sample solution: Shake thoroughly by hand each bottle of Oral Suspension. Prepare 20 µg/mL of phenobarbital from Oral Suspension and Mobile phase, and centrifuge.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 60°

Flow rate: 1.0 mL/min

Injection volume: 5 µL

System suitability

Sample: Standard solution

[NOTE—The retention time for phenobarbital is about 6.8 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of phenobarbital ($C_{12}H_{12}N_2O_3$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Phenobarbital RS in the Standard solution (µg/mL)

C_U = nominal concentration of phenobarbital in the Sample solution (µg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **pH (791):** 3.8–4.8

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.

• **BEYOND-USE DATE:** NMT 115 days after the date on which it was compounded, when stored at controlled room temperature

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the Beyond-Use Date.

- **USP REFERENCE STANDARDS** (11)
USP Phenobarbital RS

Phenobarbital Tablets

» Phenobarbital Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{12}H_{12}N_2O_3$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—
USP Phenobarbital RS

Identification—

A: Triturate a quantity of finely powdered Tablets, equivalent to about 60 mg of phenobarbital, with 50 mL of chloroform, and filter. Evaporate the clear filtrate to dryness, and dry at 105° for 2 hours; the residue so obtained responds to Identification test A under Phenobarbital.

B: The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that of the Standard preparation, both relative to the internal standard, as obtained in the Assay.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{12}H_{12}N_2O_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 240 nm on filtered portions of the solution under test, suitably diluted with pH 9.6 alkaline borate buffer (see Buffer Solutions in the section Reagents, Indicators, and Solutions), in comparison with a Standard solution having a known concentration of USP Phenobarbital RS in the same Medium.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{12}H_{12}N_2O_3$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

pH 4.5 Buffer solution, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system—Prepare as directed in the Assay under Phenobarbital.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 20 mg of phenobarbital, add 15.0 mL of Internal standard solution, mix, and sonicate for 15 minutes. Filter through a membrane filter having a 0.5- μ m or finer porosity before use.

Procedure—Proceed as directed for Procedure in the Assay under Phenobarbital. Calculate the quantity, in mg, of $C_{12}H_{12}N_2O_3$ in the portion of Tablets taken by the formula:

$$(W)(R_u / R_s)$$

in which the terms are as defined therein.

Phenobarbital Sodium

$C_{12}H_{11}N_2NaO_3$ 254.22

2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-phenyl-, monosodium salt.

Sodium 5-ethyl-5-phenylbarbiturate [57-30-7].

» Phenobarbital Sodium contains not less than 98.5 percent and not more than 101.0 percent of $C_{12}H_{11}N_2NaO_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards (11)—

USP Endotoxin RS

USP Phenobarbital RS

Completeness of solution—Mix 1.0 g with 10 mL of carbon dioxide-free water; after 1 minute, the solution is clear and free from undissolved solid.

Identification—

A: Dissolve about 50 mg of Phenobarbital Sodium in 15 mL of water in a separator, add 2 mL of hydrochloric acid, shake, and extract the liberated phenobarbital with four 25-mL portions of chloroform. Filter the combined extracts through a pledget of cotton or other suitable filter into a beaker, and wash the separator and the filter with several small portions of chloroform. Evaporate a 50-mL portion of the chloroform solution of phenobarbital on a steam bath with the aid of a current of air. Add 10 mL of ether, again evaporate, and dry the residue at 105° for 2 hours; the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Phenobarbital RS.

B: Ignite about 200 mg; the residue effervesces with acids, and responds to the tests for Sodium (191).

C: The relative retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

pH (791): between 9.2 and 10.2, in the solution prepared in the test for Completeness of solution.

Loss on drying (731)—Dry it at 150° for 4 hours; it loses not more than 7.0% of its weight.

Delete the following:

• **Heavy metals** (231)—Dissolve 2.0 g in 52 mL of water. Add slowly, with vigorous stirring, 8 mL of 1 N hydrochloric acid, and filter, discarding the first 5 mL of the filtrate. Dilute 20 mL of the subsequent filtrate with water to 25 mL; the limit is 0.003%. (Official 1-Jan-2018)

Other requirements—Where the label states that Phenobarbital Sodium is sterile, it meets the requirements for Sterility Tests (71) and for Bacterial endotoxins under Phenobarbital Sodium for Injection. Where the label states that Phenobarbital Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for Bacterial endotoxins under Phenobarbital Sodium for Injection.

Assay—

pH 4.5 Buffer solution, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system—Prepare as directed in the Assay under Phenobarbital.

Assay preparation—Transfer about 22 mg of Phenobarbital Sodium, accurately weighed, to a conical flask, add 15.0 mL of Internal standard solution, mix, and sonicate for 15 minutes. Pass through a membrane filter having a 0.5- μ m or finer porosity before use.

Procedure—Proceed as directed for Procedure in the Assay under Phenobarbital. Calculate the quantity, in mg, of

$C_{12}H_{11}N_2NaO_3$ in the portion of Phenobarbital Sodium taken by the formula:

$$(254.22 / 232.24)(W)(R_U / R_S)$$

in which 254.22 and 232.24 are the molecular weights of phenobarbital sodium and phenobarbital, respectively; and the other terms are as defined therein.

Phenobarbital Sodium Injection

» Phenobarbital Sodium Injection is a sterile solution of Phenobarbital Sodium in a suitable solvent. Phenobarbital may be substituted for the equivalent amount of Phenobarbital Sodium, for adjustment of the pH. The Injection contains the equivalent of not less than 90.0 percent and not more than 105.0 percent of the labeled amount of $C_{12}H_{11}N_2NaO_3$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

Labeling—The label indicates that the Injection is not to be used if it contains a precipitate.

USP Reference standards (11)—

USP Endotoxin RS

USP Phenobarbital RS

Identification—

A: Transfer to a separator a volume of Injection, equivalent to about 50 mg of phenobarbital sodium, add 15 mL of water, add 2 mL of hydrochloric acid, shake, and extract the liberated phenobarbital with four 25-mL portions of chloroform. Filter the combined extracts through a pledget of cotton or other suitable filter into a beaker, and wash the separator and the filter with several small portions of chloroform. Evaporate a 50-mL portion of the chloroform solution of phenobarbital on a steam bath with the aid of a current of air. Add 10 mL of ether, again evaporate, and dry the residue at 105° for 2 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Phenobarbital RS.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

Bacterial Endotoxins Test (85)—It contains not more than 0.3 USP Endotoxin Unit per mg of phenobarbital sodium.

pH (791): between 9.2 and 10.2.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products (1)*.

Assay—

pH 4.5 Buffer solution, Mobile phase, Internal standard solution, and Chromatographic system—Prepare as directed in the *Assay under Phenobarbital*.

Standard preparation—Transfer about 15 mg of USP Phenobarbital RS, accurately weighed, to a 50-mL volumetric flask, add 25 mL of *Mobile phase*, and sonicate if necessary to dissolve. Add 15.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.3 mg of USP Phenobarbital RS per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 65 mg of phenobarbital sodium, to a 100-mL volumetric flask, dilute with *Mobile*

phase to volume, and mix. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, add 15.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure in the Assay under Phenobarbital*. Calculate the quantity, in mg, of $C_{12}H_{11}N_2NaO_3$ in each mL of the Injection taken by the formula:

$$(254.22 / 232.24)(4W / V)(R_U / R_S)$$

in which 254.22 and 232.24 are the molecular weights of phenobarbital sodium and phenobarbital, respectively, V is the volume, in mL, of Injection taken, and the other terms are as defined therein.

Phenobarbital Sodium for Injection

» Phenobarbital Sodium for Injection is Phenobarbital Sodium suitable for parenteral use.

Change to read:

Packaging and storage—Preserve as described in *•Packaging and Storage Requirements (659)*, *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

USP Reference standards (11)—

USP Endotoxin RS

USP Phenobarbital RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products (1)*, *Specific Tests, Completeness and clarity of solutions*.

Bacterial Endotoxins Test (85)—It contains not more than 0.8 USP Endotoxin Unit per mg of phenobarbital sodium.

Change to read:

Other requirements—It conforms to the Definition, responds to the *Identification* tests, and meets the requirements for *Completeness of solution, pH, Loss on drying*, *• (Official 1-Jan-2018)* and *Assay under Phenobarbital Sodium*. It meets also the requirements for *Sterility Tests (71)*, *Uniformity of Dosage Units (905)*, and *Labeling (7)*, *Labels and Labeling for Injectable Products*.

Phenol



C_6H_6O

Phenol [108-95-2].

94.11

DEFINITION

Phenol contains NLT 99.0% and NMT 100.5% of phenol (C_6H_6O), calculated on the anhydrous basis. It may contain a suitable stabilizer.

IDENTIFICATION

[CAUTION—Avoid contact with skin because serious burns may result.]

- **A.**
Analysis: To a solution add bromine TS.
Acceptance criteria: A white precipitate is formed, and it dissolves at first but becomes permanent as more of the reagent is added.
- **B.**
Sample solution: 1 in 100
Analysis: To 10 mL of the *Sample solution* add 1 drop of ferric chloride TS.
Acceptance criteria: A violet color is produced.

ASSAY• **PROCEDURE**

Sample: 2 g

Titrimetric system

(See *Titrimetry* (541).)

Mode: Residual titration

Titrant: 0.1 N bromine VS

Back-titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

Analysis: Place the *Sample* in a 1000-mL volumetric flask, and dilute with water to volume. Pipet 20 mL of the solution into an iodine flask, add 30.0 mL of *Titrant*, then add 5 mL of hydrochloric acid, and immediately insert the stopper. Shake the flask repeatedly during 30 min, allow it to stand for 15 min, quickly add 5 mL of potassium iodide solution (1 in 5), taking precautions against the escape of bromine vapor, and at once insert the stopper. Shake thoroughly, remove the stopper, and rinse it and the neck of the flask with a small quantity of water so that the washing flows into the flask. Add 1 mL of chloroform, and shake the mixture. Titrate the liberated iodine with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination. Each mL of 0.1 N bromine is equivalent to 1.569 mg of phenol (C_6H_6O).

Acceptance criteria: 99.0%–100.5% on the anhydrous basis

IMPURITIES• **LIMIT OF NONVOLATILE RESIDUE**

Sample: 5 g

Analysis: Heat the *Sample* in a tared porcelain dish on a steam bath until it has evaporated, and dry the residue at 105° for 1 h.

Acceptance criteria: NMT 0.05%

SPECIFIC TESTS• **CLARITY OF SOLUTION AND REACTION**

Sample solution: 1 in 15

Acceptance criteria: The *Sample solution* is clear, and it is neutral or acid to litmus paper.

• **CONGEALING TEMPERATURE** (651): NLT 39°• **WATER DETERMINATION, Method I** (921): NMT 0.5%**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label it to indicate the name and amount of any substance added as a stabilizer.

Camphorated Phenol Topical Gel**DEFINITION**

Camphorated Phenol Topical Gel is a mixture of Camphor and Phenol in a suitable gel vehicle. It contains NLT

90.0% and NMT 110.0% of the labeled amount of camphor ($C_{10}H_{16}O$) and phenol (C_6H_6O).

IDENTIFICATION

- **A.** The retention times of the camphor and phenol peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

ASSAY• **PROCEDURE**

Internal standard solution: 10 mg/mL of *n*-dodecane in chloroform

Standard stock solution: 9.6 mg/mL of USP Phenol RS and 22.4 mg/mL of USP Camphor RS, in chloroform

Standard solution: 0.96 mg/mL of USP Phenol RS, 2.24 mg/mL of USP Camphor RS, and 1 mg/mL of *n*-dodecane in chloroform from *Standard stock solution* and *Internal standard solution*

Sample solution: Transfer 1 g of Topical Gel to a 50-mL flask. Add 5.0 mL of *Internal standard solution*, and dilute with chloroform to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m glass; packed with 100- to 120-mesh S1A, coated with 15% G44

Carrier gas: Helium

Temperatures

Detector: 200°

Column: 140°

Injection volume: 1–2 µL

System suitabilitySample: *Standard solution*

[NOTE—The relative retention times are 0.3 for phenol, 0.8 for camphor, and 1.0 for the internal standard.]

Suitability requirements

Resolution: NLT 2.0 between camphor and the internal standard, and NLT 5.0 between phenol and camphor

Relative standard deviation: NMT 2.0% of the peak response ratio of camphor and phenol to the internal standard for five consecutive injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of camphor (w/w) and phenol (w/w) in the portion of Topical Gel taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the corresponding analyte to the internal standard from the *Sample solution*

R_S = peak response ratio of the corresponding analyte to the internal standard from the *Standard solution*

C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of the appropriate analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature, avoid excessive heat, and close the cover after each use.

• **USP REFERENCE STANDARDS (11)**

USP Camphor RS
USP Phenol RS

Camphorated Phenol Topical Solution

DEFINITION

Camphorated Phenol Topical Solution is a solution of Camphor and Phenol in eucalyptus oil and Light Mineral Oil. It contains NLT 90.0% and NMT 110.0% of the labeled amount of camphor ($C_{10}H_{16}O$) and phenol (C_6H_6O).

IDENTIFICATION

- **A.** The retention times of the camphor and phenol peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

ASSAY

• **PROCEDURE**

Internal standard solution: 10 mg/mL of *n*-dodecane in chloroform

Standard stock solution: 9.6 mg/mL of USP Phenol RS and 22.4 mg/mL of USP Camphor RS, in chloroform

Standard solution: 0.96 mg/mL of USP Phenol RS, 2.24 mg/mL of USP Camphor RS, and 1 mg/mL of *n*-dodecane in chloroform from the *Standard stock solution* and *Internal standard solution*

Sample solution: Transfer 1 g of Topical Solution to a 50-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with chloroform to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m glass; packed with 100- to 120-mesh S1A, coated with 15% G44

Carrier gas: Helium

Temperatures

Detector: 200°

Column: 140°

Injection volume: 1–2 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times are 0.3 for phenol, 0.8 for camphor, and 1.0 for the internal standard.]

Suitability requirements

Resolution: NLT 2.0 between camphor and the internal standard, and NLT 5.0 between phenol and camphor

Relative standard deviation: NMT 2.0% of the peak response ratio of camphor and phenol to the internal standard for five consecutive injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of camphor (w/w) and phenol (w/w) in the portion of Topical Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of the corresponding analyte to the internal standard from the *Sample solution*

R_S = peak response ratio of the corresponding analyte to the internal standard from the *Standard solution*

C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of the appropriate analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 0.840–0.865

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature, avoid excessive heat, and close the cover after each use.
- **USP REFERENCE STANDARDS (11)**
USP Camphor RS
USP Phenol RS

Liquefied Phenol

DEFINITION

Liquefied Phenol is Phenol maintained in a liquid condition by the presence of about 10% of water. It contains NLT 89.0% by weight of C_6H_6O . It may contain a suitable stabilizer.

[CAUTION—Avoid contact with skin because serious burns may result.]

[NOTE—When phenol is to be mixed with a fixed oil, mineral oil, or white petrolatum, use crystalline Phenol, not Liquefied Phenol.]

IDENTIFICATION

- **A.** To a solution add bromine TS: a white precipitate is formed, and it dissolves at first but becomes permanent as more of the reagent is added.
- **B.** To 10 mL of a solution (1 in 100) add 1 drop of ferric chloride TS: a violet color is produced.

ASSAY

• **PROCEDURE**

Sample solution: Place 2 g of Liquefied Phenol in a 100-mL volumetric flask, and dilute with water to volume.

Analysis: Pipet 20 mL of the *Sample solution* into an iodine flask, add 30.0 mL of 0.1 N bromine VS, then add 5 mL of hydrochloric acid, and immediately insert the stopper. Shake the flask repeatedly during 30 min, allow it to stand for 15 min, add quickly 5 mL of potassium iodide solution (1 in 5), taking precautions against the escape of bromine vapor, and at once insert the stopper in the flask. Shake thoroughly, remove the stopper, and rinse it and the neck of the flask with a small quantity of water so that the washing flows into the flask. Add 1 mL of chloroform, and shake the mixture.

Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination (see *Titrimetry* (541), *Residual Titrations*). Each mL of 0.1 N bromine is equivalent to 1.569 mg of C_6H_6O .

Acceptance criteria: NLT 89.0% by weight of C_6H_6O

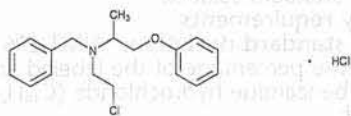
SPECIFIC TESTS

- **CLARITY OF SOLUTION AND REACTION:** A solution (1 in 15) is clear, and is neutral or acid to litmus paper.
- **LIMIT OF NONVOLATILE RESIDUE:** Heat 5 g in a tared porcelain dish on a steam bath until it has evaporated, and dry the residue at 105° for 1 h: NMT 0.05% of residue remains.
- **DISTILLING RANGE, Method I (721):** NMT 182.5°, an air-cooled condenser being used

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant glass containers.
- **LABELING:** Label it to indicate the name and amount of any substance added as a stabilizer.

Phenoxybenzamine Hydrochloride



$C_{18}H_{22}ClNO \cdot HCl$ 340.29
 Benzenemethanamine, *N*-(2-chloroethyl)-*N*-(1-methyl-2-phenoxyethyl)-, hydrochloride;
N-(2-Chloroethyl)-*N*-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride [63-92-3].

DEFINITION

Phenoxybenzamine Hydrochloride contains NLT 98.0% and NMT 101.0% of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

A. INFRARED ABSORPTION (197K)

B. ULTRAVIOLET ABSORPTION (197U)

Sample solution: 1 mg/mL of Phenoxybenzamine Hydrochloride in methanol

Analytical wavelength: 275 nm

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

ASSAY

PROCEDURE

Sample: 500 mg of Phenoxybenzamine Hydrochloride

Mode: Titrimetry

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 50 mL of glacial acetic acid, add 15 mL of mercuric acetate TS, and titrate with *Titrant*, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 34.03 mg of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$).

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

ORDINARY IMPURITIES (466)

Standard solution: Methanol

Sample solution: Methanol

Eluent: Toluene and acetone (1:1)

Visualization: 1

Acceptance criteria: Meets the requirements

SPECIFIC TESTS

MELTING RANGE OR TEMPERATURE, Class I (741):

136°–141°

LOSS ON DRYING (731)

Analysis: Dry a sample under vacuum at 60° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

PACKAGING AND STORAGE: Preserve in well-closed containers.

USP REFERENCE STANDARDS (11)

USP Phenoxybenzamine Hydrochloride RS

Phenoxybenzamine Hydrochloride Capsules

DEFINITION

Phenoxybenzamine Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$).

IDENTIFICATION

Delete the following:

A. A. ULTRAVIOLET ABSORPTION

Analytical wavelengths: 268 and 272 nm

Sample solution: 0.15 mg/mL of phenoxybenzamine hydrochloride in acidic alcohol (1 in 1000 solution of hydrochloric acid in alcohol)

Acceptance criteria: The ratio A_{268}/A_{272} of the maximum at 268 ± 2 nm and the minimum at 272 ± 2 nm is between 1.75 and 1.95. Δ USP40

Add the following:

A. A. The UV absorption spectra of the phenoxybenzamine peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak from the *Standard solution*, as obtained in the *Assay*. Δ USP40

Add the following:

A. B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. Δ USP40

ASSAY

Change to read:

PROCEDURE

Solution A: 2.2 mg/mL of anhydrous monobasic sodium phosphate in water. Adjust with Δ USP40 phosphoric acid to a pH of 3.0.

Mobile phase: Filtered and degassed mixture of *Solution A* and acetonitrile (45:55)

Standard solution: 0.2 mg/mL of USP Phenoxybenzamine Hydrochloride RS in acetonitrile. [NOTE—Sonicate if necessary.]

System suitability solution: 10 mL of the *Standard solution* and 0.5 mL of 0.1 N sodium hydroxide taken in a vial. [NOTE—Basic solutions of phenoxybenzamine hydrochloride will produce the known degradant, tertiary amine phenoxybenzamine—the second major peak that elutes before the phenoxybenzamine peak and has a relative retention time of about 0.3 and an unknown related substance. Severe degradation of the drug substance will be observed if the solution is allowed to stand for more than 1 h.]

Sample solution: Nominally 0.2 mg/mL of phenoxybenzamine hydrochloride in acetonitrile prepared as follows. Remove, as completely as possible, the contents of NLT 20 Capsules. Transfer a portion of the mixed powder, equivalent to about 10 mg of phenoxybenzamine hydrochloride, to a 50-mL volumetric flask. Add about 40 mL of acetonitrile, and sonicate for 15 min with occasional swirling. Cool, and dilute with acetoni-

trile to volume to obtain the concentration, based on the label claim. Allow the sample to stand undisturbed for 30 min such that the undissolved material settles to the bottom. Transfer the top clear solution into HPLC vials, and use as the *Sample solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

▲Detector

Assay: UV 268 nm

Identification A: Diode array, UV 240–340 nm▲USP40

Column: 4.6-mm × 150-cm; packing L7

▲USP40

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

▲[NOTE—The relative retention times for the phenoxybenzamine peak and the known degradant, tertiary amine phenoxybenzamine, peak are about 1.0 and 0.3, respectively.]▲USP40

Suitability requirements

Resolution: NLT 4 between phenoxybenzamine and the unknown peak eluting after the phenoxybenzamine peak (at about 9.4 min), *System suitability solution*

Relative standard deviation: NMT 2%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Phenoxybenzamine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phenoxybenzamine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 1: 100 rpm

Time: 45 min

Buffer: 2.2 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.00 ± 0.05.

Mobile phase: Buffer and acetonitrile (9:11)

Standard solution: 0.02 mg/mL of USP Phenoxybenzamine Hydrochloride RS in Medium

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 268 nm

Column: 4.6-mm × 150-cm; packing L7

▲USP40

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2%

Calculate the percentage of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Phenoxybenzamine Hydrochloride RS from the *Standard solution* (mg/mL)

L = label claim (mg/Capsule)

V = volume of Medium, 500 mL

Tolerances: NLT 75% (Q) of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$) is dissolved.

Change to read:

- **UNIFORMITY OF DOSAGE UNITS (905):**▲USP40 Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Solution A, Mobile phase, *Standard solution*, *System suitability solution*, *Sample solution*, *Chromatographic system*, and *System suitability*: Proceed as directed in the Assay.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of the individual impurity from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

F = relative response factor, 1.1 for phenoxybenzamine tertiary amine, and 1.0 for all other individual impurities

Acceptance criteria

Individual impurities: NMT 0.5% of phenoxybenzamine tertiary amine; NMT 0.1% of any other specified or unspecified individual impurity (degradant)

Total impurities: NMT 0.5%, includes both specified and unspecified

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• USP REFERENCE STANDARDS (11)

USP Phenoxybenzamine Hydrochloride RS

Phenoxybenzamine Hydrochloride Compounded Oral Suspension

DEFINITION

Phenoxybenzamine Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$).

Prepare Phenoxybenzamine Hydrochloride Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Phenoxybenzamine Hydrochloride powder	1 g
Corn Oil, NF, a sufficient quantity to make	100 mL

Pour the weighed *Phenoxybenzamine Hydrochloride powder* into a suitable mortar. Wet the powder with a small amount of *Corn Oil*, and triturate to make a smooth paste. Add the *Corn Oil* to make the mortar contents pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated container using the *Corn Oil* to rinse the mortar. Add sufficient *Corn Oil* to bring the preparation to final volume. Shake to mix well.

ASSAY

• PROCEDURE

Solution A: 25 mM monobasic potassium phosphate adjusted with phosphoric acid to a pH of 3.1

Mobile phase: Acetonitrile and *Solution A* (70:30)

Diluent: Prepare a mixture of 20 mL of acetonitrile and 80 mL of isopropyl alcohol in a conical flask. Add 10 g of anhydrous sodium sulfate to the flask, shake well for 1 min, and allow the sodium sulfate to precipitate to the bottom. [NOTE—The addition of anhydrous sodium sulfate removes trace amounts of water in the solvent.]

Standard solution: 0.5 mg/mL of phenoxybenzamine hydrochloride prepared from USP Phenoxybenzamine Hydrochloride RS in acetonitrile

Sample solution: Shake thoroughly each bottle of Oral Suspension. Transfer 0.5 mL of the Oral Suspension into a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix well to dissolve.

Chromatographic system
(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 15-cm; 5-μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for phenoxybenzamine hydrochloride is about 5.2 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenoxybenzamine hydrochloride ($C_{18}H_{22}ClNO \cdot HCl$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenoxybenzamine hydrochloride from the *Sample solution*

r_S = peak response of phenoxybenzamine hydrochloride from the *Standard solution*

C_S = concentration of phenoxybenzamine hydrochloride in the *Standard solution* (mg/mL)

C_U = nominal concentration of phenoxybenzamine hydrochloride in the *Sample solution* (mg/mL)

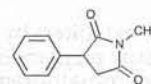
Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.

- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or controlled room temperature
- **LABELING:** Label it to indicate that it is to be well-shaken immediately before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS (11)**
USP Phenoxybenzamine Hydrochloride RS

Phensuximide



$C_{11}H_{11}NO_2$ 189.21

2,5-Pyrrolidinedione, 1-methyl-3-phenyl-, (±)-.
(±)-N-Methyl-2-phenylsuccinimide [86-34-0].

» Phensuximide contains not less than 97.0 percent and not more than 103.0 percent of $C_{11}H_{11}NO_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Phensuximide RS

Identification—

A: *Infrared Absorption* (197K).

B: *Ultraviolet Absorption* (197U)—

Solution: 400 μg per mL.

Medium: alcohol.

Melting range, Class I (741): between 68° and 74°.

Water Determination, Method I (921): not more than 1.0%.

Residue on ignition (281): not more than 0.5%.

Limit of cyanide—Dissolve 1.0 g in 10 mL of warm alcohol, and add 3 drops of ferrous sulfate TS, 1 mL of 1 N sodium hydroxide, and a few drops of ferric chloride TS. Warm gently, and finally acidify with 2 N sulfuric acid: no blue precipitate or blue color is formed within 15 minutes.

Ordinary impurities (466)—

Test solution: 200 mg of phensuximide per mL in methylene chloride.

Standard solutions: 1.0, 2.0, and 4.0 mg per mL in methylene chloride.

Application volume: 5 μL.

Eluant: a mixture of ethyl acetate and hexanes (1:1).

Visualization: 6.

Assay—Transfer about 200 mg of Phensuximide, accurately weighed, to a 50-mL volumetric flask. Dissolve in 40 mL of alcohol, dilute with alcohol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with alcohol to volume, and mix. Concomitantly determine the absorbances of this solution and of a *Standard solution* of USP Phensuximide RS, in the same medium having a known concentration of about 400 μg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 258 nm, with a suitable spectrophotometer, using alcohol as the blank. Calculate the quantity, in mg, of $C_{11}H_{11}NO_2$ in the Phensuximide taken by the formula:

$$0.5C(A_U / A_S)$$

in which C is the concentration, in μg per mL, of USP Phensuximide RS in the *Standard solution*, and A_U and A_S are the absorbances of the solution from Phensuximide and the *Standard solution*, respectively.

Phensuximide Capsules

» Phensuximide Capsules contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{11}H_{11}NO_2$.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Phensuximide RS

Identification—

A: The contents of Capsules respond to *Identification test A* under *Phensuximide*.

B: The retention time exhibited by phensuximide in the chromatogram of the *Assay preparation* corresponds to that of phensuximide in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 120 minutes.

Procedure—Determine the amount of $C_{11}H_{11}NO_2$ dissolved, employing the procedure set forth in the *Assay*, making any necessary modifications.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_{11}H_{11}NO_2$ is dissolved in 120 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Phensuximide RS in *Mobile phase* to obtain a solution having a known concentration of about 1 mg per mL.

Assay preparation—Place 10 Capsules in a 500-mL volumetric flask, and add 280 mL of water. Sonicate in a water bath at 40° to 50°, with occasional shaking, until the Capsules have broken, and cool to room temperature. Dilute with acetonitrile to volume, mix, and filter. Transfer an accurately measured volume of this specimen solution, equivalent to about 50 mg of Phensuximide, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

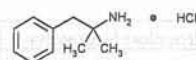
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 2100 theoretical plates, and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{11}H_{11}NO_2$ per Capsule taken by the formula:

$$2500(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Phensuximide RS in the *Standard preparation*, *V* is the volume, in mL, of specimen solution taken for the *Assay preparation*, and r_U and r_S are the phensuximide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phentermine Hydrochloride



$C_{10}H_{15}N \cdot HCl$

185.69

Benzeneethanamine, α,α -dimethyl-, hydrochloride; α,α -Dimethylphenethylamine hydrochloride [1197-21-3].

DEFINITION

Phentermine Hydrochloride contains NLT 98.0% and NMT 101.0% of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

• **B. ULTRAVIOLET ABSORPTION** (197U)

Analytical wavelength: 256 nm

Solution: 600 μ g/mL

Medium: 0.1 N hydrochloric acid

Acceptance criteria: Absorptivities at the *Analytical wavelength*, calculated on the dried basis, do not differ by more than 2.0%.

• **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191)

ASSAY

• **PROCEDURE**

Sample: About 400 mg of Phentermine Hydrochloride

Analysis: Dissolve the *Sample* in 40 mL of glacial acetic acid, and add 10 mL of mercuric acetate TS, warming slightly to effect solution. Cool to room temperature, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 18.57 mg of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$).

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **ORGANIC IMPURITIES**

Standard stock solution: 2 mg/mL of USP Phentermine Hydrochloride RS in chloroform

Standard solutions: Dilute the *Standard stock solution* with chloroform to obtain the concentrations designated in *Table 1* by letter.

Table 1

Standard solution	Dilution	Concentration (mg/mL)	Percentage (for comparison with Sample solution)
A	(undiluted)	2.0	1.0
B	(1 in 2)	1.0	0.5
C	(1 in 5)	0.4	0.2
D	(1 in 10)	0.2	0.1

Sample solution: 200 mg/mL of Phentermine Hydrochloride in chloroform

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μ L

Developing solvent system: Chloroform, cyclohexane, and diethylamine (50:40:10)

Analysis

Samples: *Standard solutions* and *Sample solution*

Position the plate in a chromatographic chamber, and develop the chromatograms in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in air. Examine the plate under short-wavelength UV light. Compare the intensities of any secondary spots observed in the *Sample solution* with those of the principal spots in the *Standard solutions*.

Acceptance criteria: The sum of the intensities of secondary spots from the *Sample solution* corresponds to NMT 1.0% of related compounds, with no single impurity corresponding to more than 0.5%.

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** (741): 202°–205°

• **pH** (791)

Sample solution: 1 in 50

Acceptance criteria: 5.0–6.0

• **LOSS ON DRYING** (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Phentermine Hydrochloride RS

Phentermine Hydrochloride Capsules**DEFINITION**

Phentermine Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$).

IDENTIFICATION

• **A.**

Sample solution: Stir a portion of the Capsule contents in acetone to prepare a solution containing a nominal concentration at about 1 mg/mL of phentermine hydrochloride.

Analysis: Filter the *Sample solution* using an acetone-resistant filter. Transfer 1 mL of the clear filtrate to a mortar containing about 200 mg of potassium bromide, triturate with a pestle, and air-dry to allow the acetone to evaporate. Place in an oven at 125° for 30 min to dry the mixture.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion prepared from the residue exhibits maxima only at the same wavelengths as that of a similar preparation of USP Phentermine Hydrochloride RS.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: 1.1 g of sodium 1-heptanesulfonate in 575 mL of water. Add 25 mL of dilute glacial acetic acid (14 in 100) and 400 mL of methanol. Adjust dropwise, if necessary, with glacial acetic acid to a pH of 3.3 ± 0.1 . Pass through a membrane filter of 0.5- μ m pore size. The volume of methanol may be adjusted to provide a suitable retention time for phentermine hydrochloride (about 8 min).

Diluent: 0.04 M phosphoric acid

Standard solution: 0.4 mg/mL of USP Phentermine Hydrochloride RS in *Diluent*

Sample solution: Remove, as completely as possible, the contents of NLT 20 Capsules, and weigh. Transfer a portion of the mixed powder, nominally equivalent to about 20 mg of phentermine hydrochloride, to a 50-mL volumetric flask. Add 40 mL of *Diluent*, and sonicate for 15 min. Dilute with *Diluent* to volume, and mix. Pass through a membrane filter of 0.5- μ m pore size, discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 50 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Phentermine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phentermine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION**, *Procedure for a Pooled Sample* (711)

Medium: Water; 900 mL. Use 500 mL for Capsules containing 15 mg or less of phentermine hydrochloride.

Apparatus 2: 50 rpm

Time: 45 min

Analysis: Determine the amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$) dissolved, by using the *Procedure* set forth in the *Assay*, making any necessary modifications including concentration of the analyte in the volume of the *Sample solution* taken.

Tolerances: NLT 75% (Q) of the labeled amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Phentermine Hydrochloride RS

Phentermine Hydrochloride Tablets**DEFINITION**

Phentermine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of phentermine hydrochloride ($C_{10}H_{15}N \cdot HCl$).

IDENTIFICATION

• **A.**

Sample solution: Stir a portion of finely powdered Tablet contents in acetone to prepare a solution containing a nominal concentration at about 1 mg/mL of phentermine hydrochloride.

Analysis: Filter the *Sample solution* using an acetone resistant filter. Transfer 1 mL of the clear filtrate to a mortar containing about 200 mg of potassium bromide, triturate with a pestle, and air-dry to allow the acetone to evaporate. Place in an oven at 125° for 30 min to dry the mixture.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion prepared from the residue exhibits maxima only at the same wavelengths as that of a similar preparation of USP Phentermine Hydrochloride RS.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Mobile phase: Prepare a suitably degassed solution containing 0.03% diethylamine in methanol.

Internal standard solution: About 0.02 mg/mL of caffeine in *Mobile phase*.

Standard solution: USP Phentermine Hydrochloride RS in the *Internal standard solution*, equivalent to 0.75 mg/mL of phentermine hydrochloride.

Sample solution: Transfer an equivalent to 7.5 mg, from NLT 20 finely powdered Tablets, to a suitable flask. Pipet 10.0 mL of the *Internal standard solution* into the flask. Insert the stopper, mix, and sonicate for about 10 min. Pass through a filter of 0.5-μm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for caffeine and phentermine are about 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4 between caffeine and phentermine

Column efficiency: NLT 2000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phentermine hydrochloride (C₁₀H₁₅N · HCl) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of phentermine to the internal standard from the *Sample solution*

R_S = peak response ratio of phentermine to the internal standard from the *Standard solution*

C_S = concentration of USP Phentermine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phentermine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION, Procedure for a Pooled Sample (711)

Medium: Water; 900 mL. Use 500 mL for Tablets containing 15 mg or less of phentermine hydrochloride.

Apparatus 2: 50 rpm

Time: 45 min

Solution A: Dissolve 1.1 g of sodium 1-heptanesulfonate in 1 L of water. Add 3.5 mL of glacial acetic acid, and mix.

Mobile phase: Methanol and *Solution A* (525:475). Filter, degas, and adjust with phosphoric acid to a pH of 2.5.

Sample solution: Filtered portion of the pooled sample under test

Standard solution: Dissolve USP Phentermine Hydrochloride RS in water, and dilute with water, if necessary, to obtain a known concentration approximately equivalent to the *Sample solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 208 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 25 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of the labeled amount of phentermine hydrochloride (C₁₀H₁₅N · HCl) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L)$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Phentermine Hydrochloride RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL or 500 mL

L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of phentermine hydrochloride (C₁₀H₁₅N · HCl) is dissolved.

UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Sample solution: Proceed as directed in the *Assay*, except prepare the *Sample solution* as follows. Transfer 1 Tablet to each of 10 suitable containers, and add 1 mL of water and 10 mL of the *Internal standard solution* to each. Mix, sonicate for about 10 min after each Tablet has disintegrated, and filter.

Acceptance criteria: Meet the requirements

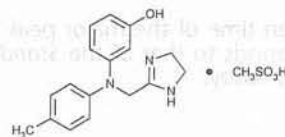
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Phentermine Hydrochloride RS

Phentolamine Mesylate



C₁₇H₁₉N₃O · CH₄O₃S 377.46

Phenol, 3-[[[4,5-dihydro-1H-imidazol-2-yl)methyl](4-methylphenyl)amino]-, monomethanesulfonate (salt).

m-[N-(2-Imidazolin-2-ylmethyl)-*p*-toluidino]phenol monomethanesulfonate (salt) [65-28-1].

» Phentolamine Mesylate contains not less than 98.0 percent and not more than 102.0 percent of C₁₇H₁₉N₃O · CH₄O₃S, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards (11)—
USP Phentolamine Mesylate RS

Identification—

A: Infrared Absorption (197M).

B: Ultraviolet Absorption (197U)—

Solution: 20 µg per mL.

Medium: water.

C: The R_f value of the principal spot in the chromatogram of the *Identification preparation* corresponds to that of *Standard preparation A* as obtained in the test for *Chromatographic purity*.

Loss on drying (731)—Dry it in vacuum at 60° for 4 hours; it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.1%.

Sulfate (221)—A 0.10-g portion shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.2%).

Chromatographic purity—

Standard preparations—Dissolve USP Phentolamine Mesylate RS in methanol, and mix to obtain *Standard preparation A* having a known concentration of 50 µg per mL. Quantitatively dilute with methanol to obtain *Standard preparations*, designated below by letter, having the following compositions:

Standard preparation	Dilution	Concentration (µg RS per mL)	Percentage (% for comparison with test specimen)
A	(undiluted)	50	0.5
B	(3 in 5)	30	0.3
C	(1 in 5)	10	0.1

Test preparation—Dissolve an accurately weighed quantity of Phentolamine Mesylate in methanol to obtain a solution containing 10 mg per mL.

Identification preparation—Dilute a portion of the *Test preparation* quantitatively with methanol to obtain a solution containing 50 µg per mL.

Detection reagent—Prepare (1) a solution of 1 g of potassium ferricyanide in 20 mL of water, and (2) a solution of 1.9 g of ferric chloride in 20 mL of water. Just prior to use, mix equal volumes of the solutions.

Procedure—Apply separately 5 µL of the *Test preparation*, 5 µL of the *Identification preparation*, and 5 µL of each *Standard preparation* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel, and allow to dry. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of chloroform, diethylamine, and methanol (15:3:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 100° for 1 hour. Spray the plate with *Detection reagent*. Within 15 minutes after spraying, compare the intensities of any secondary spots observed in the chromatogram of the *Test preparation* with those of the principal spots in the chromatograms of the *Standard preparations*: no secondary spot from the chromatogram of the *Test preparation* is larger or more intense than the principal spot obtained from *Standard preparation A* (0.5%), and the sum of the intensities of all secondary spots obtained from the *Test preparation* corresponds to not more than 1.0%.

Assay—

0.1 N Tetrabutylammonium hydroxide in isopropyl alcohol—Dilute with dehydrated isopropyl alcohol a commercially available 25% solution of tetrabutylammonium hydroxide in methanol, and standardize as directed under *Tetrabutylammonium Hydroxide, Tenth-Normal* (0.1 N) (see *Volumetric Solutions* in the section *Reagents, Indicators, and Solutions*), using dehydrated isopropyl alcohol instead of dimethylformamide.

Procedure—Dissolve with the aid of sonication, if necessary, about 300 mg of Phentolamine Mesylate, accurately weighed, in 100 mL of dehydrated isopropyl alcohol. Titrate in an atmosphere of nitrogen with 0.1 N Tetrabutylammonium hydroxide in isopropyl alcohol, determining the endpoint potentiometrically, using a glass electrode and a calomel electrode containing a saturated solution of tetramethylammonium chloride in dehydrated isopropyl alcohol (see *Titrimetry* (541)). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 37.75 mg of $C_{17}H_{19}N_3O \cdot CH_4O_3S$.

Phentolamine Mesylate for Injection

» Phentolamine Mesylate for Injection is sterile Phentolamine Mesylate or a sterile mixture of Phentolamine Mesylate with a suitable buffer or suitable diluents. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{19}N_3O \cdot CH_4O_3S$.

Change to read:

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

USP Reference standards (11)—

USP Endotoxin RS

USP Phentolamine Mesylate RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

Identification—Mix a portion of it, equivalent to about 40 mg of phentolamine mesylate, with about 15 mL of chloroform. Filter into a beaker, and evaporate to dryness, taking precautions against introducing moisture: the residue so obtained responds to *Identification test A* under *Phentolamine Mesylate*.

Bacterial Endotoxins Test (85)—It contains not more than 5.8 USP Endotoxin Units per mg of phentolamine mesylate.

Uniformity of dosage units (905): meets the requirements.

Procedure for content uniformity—Dissolve the contents of 1 container in water to provide a solution containing about 20 µg of phentolamine mesylate per mL. Concomitantly determine the absorbances of this solution and of a solution of USP Phentolamine Mesylate RS, in the same medium, at a concentration of about 20 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 278 nm, with a suitable spectrophotometer, using water as the blank. Cal-

culate the quantity, in mg, of $C_{17}H_{19}N_3O \cdot CH_4O_3S$ in the Phentolamine Mesylate for Injection taken by the formula:

$$(T/D)C(A_U/A_S)$$

in which T is the labeled quantity, in mg, of phentolamine mesylate in the Phentolamine Mesylate for Injection, D is the concentration, in μg per mL, of phentolamine mesylate in the solution from the Phentolamine Mesylate for Injection, based on the labeled quantity per container and the extent of dilution, C is the concentration, in μg per mL, of USP Phentolamine Mesylate RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Phentolamine Mesylate for Injection and the Standard solution, respectively.

pH (791): between 4.5 and 6.5, in a freshly prepared solution having a concentration of about 1 in 100.

Other requirements—It meets the requirements for *Sterility Tests* (71) and *Labeling* (7), *Labels and Labeling for Injectable Products*.

Assay—

Standard preparation—Transfer about 25 mg of USP Phentolamine Mesylate RS, accurately weighed, to a 50-mL volumetric flask, add water to volume, and mix.

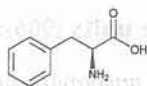
Assay preparation—Dissolve the contents of 10 containers of Phentolamine Mesylate for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Transfer an aliquot, equivalent to about 25 mg of phentolamine mesylate, to a 50-mL volumetric flask, add water to volume, and mix.

Procedure—Pipet 5-mL portions, respectively, of the *Standard preparation*, *Assay preparation*, and water to provide a blank, into separate 125-mL separators. Into each separator pipet 5-mL portions of 0.1 N hydrochloric acid and saturated picric acid solution. Extract with three 25-mL portions of chloroform, filtering each portion through chloroform-washed cotton into a 100-mL volumetric flask. Dilute with chloroform to volume, and mix. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 410 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_{17}H_{19}N_3O \cdot CH_4O_3S$ in the aliquot of Phentolamine Mesylate for Injection taken by the formula:

$$50C(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Phentolamine Mesylate RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Phenylalanine



$C_9H_{11}NO_2$ 165.19
L-Phenylalanine [63-91-2].

DEFINITION

Phenylalanine contains NLT 98.5% and NMT 101.5% of L-phenylalanine ($C_9H_{11}NO_2$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

ASSAY

• PROCEDURE

Sample: 160 mg of Phenylalanine

Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with the *Titrant*. Perform the *Blank* determination.

Calculate the percentage of phenylalanine ($C_9H_{11}NO_2$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 165.2 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.4%

- **CHLORIDE AND SULFATE, Chloride** (221)

Standard solution: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.73 g of Phenylalanine

Acceptance criteria: NMT 0.05%

- **CHLORIDE AND SULFATE, Sulfate** (221)

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.33 g of Phenylalanine

Acceptance criteria: NMT 0.03%

- **IRON** (241): NMT 30 ppm

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 15 ppm (Official 1-

Jan-2018)

- **RELATED COMPOUNDS**

Diluent: Glacial acetic acid and water (1:1)

Standard solution: 0.05 mg/mL of USP

L-Phenylalanine RS in *Diluent*. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample* solution.]

System suitability solution: 0.4 mg/mL each of USP

L-Phenylalanine RS and USP L-Tyrosine RS in *Diluent*

Sample solution: 10 mg/mL of Phenylalanine in *Diluent*

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μL

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)

Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

System suitability

Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

Analysis

Samples: *Standard solution*, *System suitability solution*, and *Sample solution*

After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.

Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

• **OPTICAL ROTATION, Specific Rotation (781S)**

Sample solution: 20 mg/mL in water

Acceptance criteria: -32.7° to -34.7°

• **pH (791)**

Sample solution: 10 mg/mL solution

Acceptance criteria: 5.4–6.0

• **LOSS ON DRYING (731):** Dry a sample at 105° for 3 h: it loses NMT 0.3% of its weight.

ADDITIONAL REQUIREMENTS

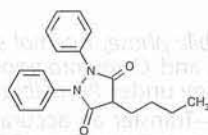
• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**

USP L-Phenylalanine RS

USP L-Tyrosine RS

Phenylbutazone



$C_{19}H_{20}N_2O_2$ 308.37

3,5-Pyrazolidinedione, 4-butyl-1,2-diphenyl-

4-Butyl-1,2-diphenyl-3,5-pyrazolidinedione [50-33-9].

» Phenylbutazone contains not less than 98.0 percent and not more than 102.0 percent of $C_{19}H_{20}N_2O_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Phenylbutazone RS

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 10 μ g per mL.

Medium: sodium hydroxide solution (1 in 2500).

Absorptivities at 264 nm, calculated on the dried basis, do not differ by more than 2.0%.

Melting range (741): between 104° and 107° .

Loss on drying (731)—Dry it in vacuum at a pressure of 30 ± 10 mm of mercury at 80° for 4 hours: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.1%, 2.0 g being used for the test.

Chloride (221)—Boil 2.0 g with 60 mL of water for 5 minutes, cool, and filter. To a 30-mL portion of the filtrate add 1 mL of 2 N nitric acid and 1 mL of silver nitrate TS: the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.007%).

Sulfate (221)—To a 30-mL portion of the filtrate obtained in the test for *Chloride* add 2 mL of barium chloride TS: the mixture shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.01%).

Delete the following:

• **Heavy metals, Method II (231):** 0.001%. • (Official 1-Jan-2018)

Assay—

Acetate buffer—Transfer 2.72 g of sodium acetate to a 1000-mL beaker, and dissolve in about 700 mL of water. Adjust with glacial acetic acid to a pH of 4.1. Filter through a 0.5- μ m filter, dilute with filtered water to 1000 mL, and mix.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile with 560 mL of *Acetate buffer* (440:560). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard solution—Dissolve 300 mg of desoxycorticosterone acetate in 200 mL of acetonitrile, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Phenylbutazone RS in acetonitrile, with the aid of sonication, and dilute quantitatively with acetonitrile to obtain a solution having a concentration of about 1.4 mg per mL. Pipet 10 mL of this solution into a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with acetonitrile to volume, and mix. [NOTE—Use this solution within 8 hours of its preparation.]

Assay preparation—Transfer about 140 mg of Phenylbutazone, accurately weighed, to a 100-mL volumetric flask, add 75 mL of acetonitrile, and sonicate to dissolve. Dilute with acetonitrile to volume, and mix. Pipet 10 mL of this solution into a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with acetonitrile to volume, and mix. [NOTE—Use this solution within 8 hours of its preparation.]

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm \times 25-cm column that contains packing L7, preceded by a pre-column that contains packing L2. The flow rate is about 2.4 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, R , of phenylbutazone and the internal standard is not less than 3.5, and the relative standard deviation of the ratio of their peak responses in replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 1.0 for the internal standard and 0.7 for phenylbutazone. Calculate the quantity, in mg, of $C_{19}H_{20}N_2O_2$ in the portion of Phenylbutazone taken by the formula:

$$500C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Phenylbutazone RS in the *Standard preparation*; and R_U and R_S are the ratios of the peak response of the phenylbutazone to that of the internal standard for the *Assay preparation* and the *Standard preparation*, respectively.

Phenylbutazone Boluses

» Phenylbutazone Boluses contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of phenylbutazone ($C_{19}H_{20}N_2O_2$) and nominally not less than 1 g of phenylbutazone per bolus.

Packaging and storage—Preserve in well-closed containers.

Labeling—Label Boluses to indicate that they are for veterinary use only.

USP Reference standards (11)—

USP Phenylbutazone RS

Identification—

A: Transfer a portion of powdered Boluses, equivalent to about 500 mg of phenylbutazone, to a 250-mL conical flask, add 100 mL of solvent hexane, and heat the mixture under reflux for 15 minutes. Filter the hot mixture, and allow the filtrate to cool. Separate the crystals thus formed by filtration, and dry in vacuum at 80° for 30 minutes: the phenylbutazone so obtained responds to *Identification test A* under *Phenylbutazone*.

B: The retention time of the phenylbutazone peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Disintegration (701): 45 minutes with disks, determined as directed for *Uncoated Tablets*, simulated gastric fluid being used as the immersion fluid.

Uniformity of dosage units (905)—meet the requirements for *Weight Variation*.

Assay—

Acetate buffer, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay* under *Phenylbutazone*.

Assay preparation—Weigh and finely powder a Phenylbutazone Bolus. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of phenylbutazone, to a 250-mL volumetric flask. Transfer 10.0 mL of water to the flask, and shake by mechanical means for 15 minutes. Add about 120 mL of acetonitrile, and sonicate until insoluble material is dispersed into fine particles. Shake by mechanical means for 20 minutes, dilute with acetonitrile to volume, and mix. Transfer 7.0 mL of this solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with acetonitrile to volume, and mix. Pass a portion of this solution through a filter having a porosity of 0.5 µm or finer, discarding the first few mL of the filtrate. Use the clear filtrate as the *Assay preparation*. [NOTE—Use this solution within 8 hours of its preparation.]

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Phenylbutazone*. Calculate the quantity, in mg, of $C_{19}H_{20}N_2O_2$ in the portion of the Bolus taken by the formula:

$$1786C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Phenylbutazone RS in the *Standard preparation*, and R_U and R_S are the ratios of the peak responses of phenylbutazone to that of the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenylbutazone Injection

» Phenylbutazone Injection is a sterile solution of Phenylbutazone in Sterile Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{19}H_{20}N_2O_2$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Protect from light, and store in a refrigerator.

Labeling—Label Injection to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Phenylbutazone RS

Clarity of solution—The Injection is essentially free from particles of foreign matter that can be observed on visual inspection.

Identification—

A: Transfer a volume of Injection, equivalent to about 500 mg of phenylbutazone, to a 250-mL conical flask, add 100 mL of solvent hexane, and heat the mixture under reflux for 15 minutes. Filter the hot mixture, and allow the filtrate to cool. Separate the crystals thus formed by filtration, and dry in vacuum at 80° for 30 minutes: the phenylbutazone so obtained responds to *Identification test A* under *Phenylbutazone*.

B: The retention time of the phenylbutazone peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

Bacterial Endotoxins Test (85)—It contains not more than 1.1 USP Endotoxin Units per mg of phenylbutazone.

pH (791): between 9.5 and 10.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Acetate buffer, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay* under *Phenylbutazone*.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 200 mg of phenylbutazone, to a 100-mL volumetric flask. Dilute with acetonitrile to volume, and mix. Transfer 7.0 mL of this solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with acetonitrile to volume, and mix. [NOTE—Use this solution within 8 hours of its preparation.]

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Phenylbutazone*. Calculate the quantity, in mg, of $C_{19}H_{20}N_2O_2$ in each mL of the Injection taken by the formula:

$$350(C/V)(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Phenylbutazone RS in the *Standard preparation*, *V* is the volume, in mL, of Injection taken to prepare the *Assay preparation*, and R_U and R_S are the ratios of the peak responses of phenylbutazone to that of the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenylbutazone Tablets

» Phenylbutazone Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of phenylbutazone ($C_{19}H_{20}N_2O_2$) and nominally not more than 200 mg of phenylbutazone per Tablet.

Packaging and storage—Preserve in tight containers.

Labeling—Label Tablets to indicate that they are for veterinary use only.

USP Reference standards (11)—

USP Phenylbutazone RS

Identification—Transfer to a 250-mL conical flask a portion of powdered Tablets, equivalent to about 500 mg of

phenylbutazone, add 100 mL of solvent hexane, and heat the mixture under reflux for 15 minutes. Filter the hot mixture, and allow the filtrate to cool. Separate the crystals thus formed by filtration, and dry in vacuum at 80° for 30 minutes: the phenylbutazone so obtained responds to *Identification test A* under *Phenylbutazone*.

Dissolution (711)—

Medium: pH 7.5 simulated intestinal fluid TS (without the enzyme); 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{19}H_{20}N_2O_2$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 264 nm on filtered portions of the solution under test, suitably diluted, if necessary, with **Medium**, using a suitable spectrophotometer, 1-cm cells, and **Medium** as the blank, in comparison with a solution of known concentration of USP Phenylbutazone RS in the same **Medium**.

Tolerances—Not less than 70% (*Q*) is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Procedure for content uniformity—Transfer 1 Tablet to a 100-mL volumetric flask, add 60 mL of methanol, and shake by mechanical means for about 20 minutes or until the tablet is completely disintegrated. Dilute with methanol to volume, and mix. Filter a portion of mixture, discarding the first 10 mL of the filtrate. Dilute an accurately measured portion of the filtrate with sodium hydroxide solution (1 in 2500) to obtain a solution containing about 10 µg per mL. Prepare a solution of USP Phenylbutazone RS in methanol having a known concentration of about 1 mg per mL. Quantitatively dilute a portion of this solution with sodium hydroxide solution (1 in 2500) to obtain a Standard solution having a final known concentration of about 10 µg per mL. Concomitantly determine the absorbances of the solution from the Tablet and the Standard solution at the wavelength of maximum absorbance at about 264 nm with a suitable spectrophotometer, using sodium hydroxide solution (1 in 2500) as the blank. Calculate the quantity, in mg, of $C_{19}H_{20}N_2O_2$ in the Tablet by the formula:

$$(TC/D)(A_U / A_S)$$

in which *T* is the labeled quantity, in mg, of phenylbutazone in the Tablet; *C* is the concentration, in µg per mL, of USP Phenylbutazone RS in the Standard solution; *D* is the concentration, in µg per mL, of phenylbutazone in the solution from the Tablet based on the labeled quantity per Tablet and the extent of dilution; and *A_U* and *A_S* are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—

Acetate buffer, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system—Proceed as directed in the Assay under *Phenylbutazone*.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Accurately weigh a portion of the powder, equivalent to about 500 mg of phenylbutazone, and transfer to a 250-mL volumetric flask. Pipet 50 mL of water into the flask, and shake by mechanical means for 15 minutes. Add about 120 mL of acetonitrile, and sonicate until insoluble material is dispersed into fine particles. Shake by mechanical means for 20 minutes, dilute with acetonitrile to volume, and mix. Centrifuge a portion of this solution. Pipet 7 mL of the solution into a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with acetonitrile to volume, and mix. Pass a portion through a 0.5-µm filter, discarding

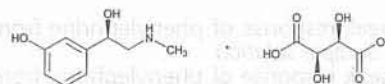
the first few mL of the filtrate. [NOTE—Use this solution within 8 hours of its preparation.]

Procedure—Proceed as directed for *Procedure* in the Assay under *Phenylbutazone*. Calculate the quantity, in mg, of phenylbutazone ($C_{19}H_{20}N_2O_2$) in the portion of Tablets taken by the formula:

$$1786C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Phenylbutazone RS in the *Standard preparation*; and *R_U* and *R_S* are the ratios of the peak response of phenylbutazone to that of the internal standard for the *Assay preparation* and the *Standard preparation*, respectively.

Phenylephrine Bitartrate



$C_9H_{13}NO_2 \cdot C_4H_6O_6$ 317.29
Benzenemethanol, 3-hydroxy-α-[(methylamino)methyl]-, (2*R*,3*R*)-2,3-dihydroxybutanedioate (1:1) (salt);
(*R*)-(-)-*m*-Hydroxy-α-[(methylamino)methyl]benzyl alcohol hydrogen tartrate [17162-39-9].

DEFINITION

Phenylephrine Bitartrate contains NLT 98.0% and NMT 102.0% of phenylephrine bitartrate ($C_9H_{13}NO_2 \cdot C_4H_6O_6$), calculated on the dried basis.

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

• B. IDENTIFICATION TESTS—GENERAL, Tartrate (191)

Sample: The alkaline filtrate from the test for *Optical Rotation* (781S), *Specific Rotation*

Acceptance criteria: The *Sample* responds positively to the test for *Tartrate* in *Identification Tests—General* (191).

C. The retention time of the major peak of the *Sample* solution corresponds to that of the *Standard* solution, as obtained in the Assay.

ASSAY

• PROCEDURE

Buffer: Dissolve 3.25 g of 1-octanesulfonic acid sodium salt monohydrate in 1 L of water, and adjust with 3 M phosphoric acid to a pH of 2.8.

Solution A: Acetonitrile and *Buffer* (10:90)

Solution B: Acetonitrile and *Buffer* (90:10)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93	7
10	70	30
10.1	93	7
18	93	7

Diluent: *Solution A* and *Solution B* (80:20)

Standard solution: 0.6 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*

Sample solution: 0.9 mg/mL of Phenylephrine Bitartrate in *Diluent*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.0-mm × 5.5-cm; 3-μm packing L1

Column temperature: 45°

Flow rate: 1.5 mL/min

Injection volume: 4 μL

System suitabilitySample: *Standard solution***Suitability requirements**

Tailing factor: NMT 1.9

Relative standard deviation: NMT 0.73%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of phenylephrine bitartrate ($C_9H_{13}NO_2 \cdot C_4H_6O_6$) in the portion of Phenylephrine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of phenylephrine from the *Sample solution* r_S = peak response of phenylephrine from the *Standard solution* C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = concentration of Phenylephrine Bitartrate in the *Sample solution* (mg/mL) M_{r1} = molecular weight of phenylephrine bitartrate, 317.29 M_{r2} = molecular weight of phenylephrine hydrochloride, 203.67

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES• **RESIDUE ON IGNITION** (281): NMT 0.1%• **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, and Diluent: Proceed as directed in the Assay.

System suitability solution: 1.0 mg/mL of USP Phenylephrine Hydrochloride RS and 0.9 μg/mL each of USP Norphenylephrine Hydrochloride RS and USP Phenylephrine Related Compound C RS in *Diluent*Standard solution: 0.001 mg/mL each of USP Phenylephrine Hydrochloride RS, USP Norphenylephrine Hydrochloride RS, USP Phenylephrine Related Compound C RS, USP Phenylephrine Related Compound D RS, and USP Phenylephrine Related Compound E RS in *Diluent*Blank: 0.8 mg/mL of L(+)-tartaric acid in *Diluent*Sample solution: 1.56 mg/mL of Phenylephrine Bitartrate in *Diluent***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4-mm × 5.5-cm; 3-μm packing L1

Column temperature: 45°

Flow rate: 1.5 mL/min

Injection volume: 4 μL

System suitabilitySamples: *System suitability solution* and *Standard solution***Suitability requirements**Resolution: NLT 1.5 between norphenylephrine and phenylephrine and NLT 1.5 between phenylephrine and phenylephrine related compound C, *System suitability solution*Relative standard deviation: NMT 5% for norphenylephrine, phenylephrine, phenylephrine related compound C, phenylephrine related compound D, and phenylephrine related compound E, *Standard solution***Analysis**Samples: *Standard solution*, *Blank*, and *Sample solution*
Examine the chromatogram of the *Blank* for the peaks, and disregard any corresponding peaks observed in the chromatogram of the *Sample solution*.

Calculate the percentage of norphenylephrine as free base in the portion of Phenylephrine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of norphenylephrine from the *Sample solution* r_S = peak response of norphenylephrine from the *Standard solution* C_S = concentration of USP Norphenylephrine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = concentration of Phenylephrine Bitartrate in the *Sample solution* (mg/mL) M_{r1} = molecular weight of norphenylephrine as free base, 153.18 M_{r2} = molecular weight of norphenylephrine as hydrochloride salt, 189.64

Calculate the percentage of phenylephrine related compound C as free base in the portion of Phenylephrine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of phenylephrine related compound C from the *Sample solution* r_S = peak response of phenylephrine related compound C from the *Standard solution* C_S = concentration of USP Phenylephrine Related Compound C RS in the *Standard solution* (mg/mL) C_U = concentration of Phenylephrine Bitartrate in the *Sample solution* (mg/mL) M_{r1} = molecular weight of phenylephrine related compound C as free base, 165.19 M_{r2} = molecular weight of phenylephrine related compound C as hydrochloride salt, 201.65

Calculate the percentage of phenylephrine related compound D in the portion of Phenylephrine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of phenylephrine related compound D from the *Sample solution* r_S = peak response of phenylephrine related compound D from the *Standard solution* C_S = concentration of USP Phenylephrine Related Compound D RS in the *Standard solution* (mg/mL) C_U = concentration of Phenylephrine Bitartrate in the *Sample solution* (mg/mL)

Calculate the percentage of phenylephrine related compound E as free base in the portion of Phenylephrine Bitartrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of phenylephrine related compound E from the *Sample solution* r_S = peak response of phenylephrine related compound E from the *Standard solution* C_S = concentration of USP Phenylephrine Related Compound E RS in the *Standard solution* (mg/mL) C_U = concentration of Phenylephrine Bitartrate in the *Sample solution* (mg/mL) M_{r1} = molecular weight of phenylephrine related compound E as free base, 255.31 M_{r2} = molecular weight of phenylephrine related compound E as hydrochloride salt, 291.77

Calculate the percentage of any individual unspecified impurity in the portion of Phenylephrine Bitartrate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

r_u = peak response of each unspecified impurity from the *Sample solution*

r_s = peak response of phenylephrine from the *Standard solution*

C_s = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)

C_u = concentration of Phenylephrine Bitartrate in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of phenylephrine bitartrate, 317.29

M_{r2} = molecular weight of phenylephrine hydrochloride, 203.67

Acceptance criteria: See Table 2. Disregard any peaks below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Norphenylephrine	0.9	0.2
Phenylephrine	1.0	—
Phenylephrine related compound C	1.2	0.1
Phenylephrine related compound D	2.9	0.2
Phenylephrine related compound E	3.1	0.1
Any individual unspecified impurity	—	0.1
Total impurities	—	0.5

SPECIFIC TESTS

• OPTICAL ROTATION, Specific Rotation (781S)

Sample solution: Prepare a solution of about 240 mg/mL of Phenylephrine Bitartrate in water. Make the solution slightly alkaline by adding concentrated ammonium hydroxide dropwise. Rub the wall of the vessel with a glass rod so that the base precipitates out. Filter the base under suction, wash with a little water and acetone, and dry at 105° for 2 h. Prepare and measure a 50-mg/mL solution of base precipitate in 1 M hydrochloric acid.

Acceptance criteria: −53° to −57°

• pH (791)

Sample solution: 10% w/v aqueous solution

Acceptance criteria: 3.0–4.0

• LOSS ON DRYING (731)

Analysis: Dry at 105° to a constant weight.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in tight, light-resistant containers. Store at controlled room temperature.

• USP REFERENCE STANDARDS (11)

USP Norphenylephrine Hydrochloride RS

3-(2-Amino-1-hydroxyethyl)phenol hydrochloride.

$C_8H_{11}NO_2 \cdot HCl$ 189.64

USP Phenylephrine Bitartrate RS

USP Phenylephrine Hydrochloride RS

USP Phenylephrine Related Compound C RS

1-(3-Hydroxyphenyl)-2-(methylamino)ethan-1-one hydrochloride.

$C_9H_{11}NO_2 \cdot HCl$ 201.65

USP Phenylephrine Related Compound D RS

(R)-3-[2-[Benzyl(methyl)amino]-1-hydroxyethyl]phenol.

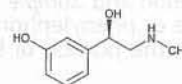
$C_{16}H_{19}NO_2$ 257.33

USP Phenylephrine Related Compound E RS

2-[Benzyl(methyl)amino]-1-(3-hydroxyphenyl)ethan-1-one hydrochloride.

$C_{16}H_{17}NO_2 \cdot HCl$ 291.77

Phenylephrine Hydrochloride



$C_9H_{13}NO_2 \cdot HCl$

203.67

Benzenemethanol, 3-hydroxy- α -[(methylamino)methyl]-, hydrochloride (R)-;

(-)-*m*-Hydroxy- α -[(methylamino)methyl]benzyl alcohol hydrochloride [61-76-7].

DEFINITION

Phenylephrine Hydrochloride contains NLT 98.0% and NMT 102.0% of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

• B. IDENTIFICATION TESTS—GENERAL, Chloride (191)

Sample solution: 10 mg/mL

Acceptance criteria: Meets the requirements

• C. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• PROCEDURE

Buffer: Dissolve 3.25 g of 1-octanesulfonic acid sodium salt monohydrate in 1 L of water, and adjust with 3 M phosphoric acid to a pH of 2.8.

Solution A: Acetonitrile and *Buffer* (10:90)

Solution B: Acetonitrile and *Buffer* (90:10)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	93	7
3	93	7
13	70	30
14	93	7
16	93	7

Diluent: *Solution A* and *Solution B* (80:20)

Standard solution: 0.4 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*

Sample solution: 0.4 mg/mL of Phenylephrine Hydrochloride in *Diluent*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 215 nm**Column:** 4.0-mm × 5.5-cm; 3-μm packing L1**Column temperature:** 45°**Flow rate:** 1.5 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.9**Relative standard deviation:** NMT 0.73%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) in the portion of Phenylephrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of phenylephrine from the *Sample solution* r_S = peak response of phenylephrine from the *Standard solution* C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = concentration of Phenylephrine Hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the dried basis**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.2%

- **CHLORIDE AND SULFATE**, *Sulfate* (221)

Standard solution: 0.10 mL of 0.020 N sulfuric acid**Sample solution:** 50 mg in 25 mL of water**Acceptance criteria:** The *Sample solution* shows no more turbidity than corresponds to that of the *Standard solution* (0.20%).

- **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.**System suitability solution:** 1.0 mg/mL of USP Phenylephrine Hydrochloride RS and 10 μg/mL each of USP Norphenylephrine Hydrochloride RS and USP Phenylephrine Related Compound C RS in *Diluent***Standard solution:** 0.001 mg/mL each of USP Phenylephrine Hydrochloride RS, USP Norphenylephrine Hydrochloride RS, USP Phenylephrine Related Compound C RS, USP Phenylephrine Related Compound D RS, and USP Phenylephrine Related Compound E RS in *Diluent***Sample solution:** 1.0 mg/mL of Phenylephrine Hydrochloride in *Diluent***System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 1.5 between norphenylephrine and phenylephrine and NLT 1.5 between phenylephrine and phenylephrine related compound C, *System suitability solution***Relative standard deviation:** NMT 5% for norphenylephrine, phenylephrine, phenylephrine related compound C, phenylephrine related compound D, and phenylephrine related compound E, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of norphenylephrine as free base in the portion of Phenylephrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of norphenylephrine from the *Sample solution* r_S = peak response of norphenylephrine from the *Standard solution* C_S = concentration of USP Norphenylephrine Hydrochloride RS in the *Standard solution* (mg/mL) C_U = concentration of Phenylephrine Hydrochloride in the *Sample solution* (mg/mL) M_{r1} = molecular weight of norphenylephrine as free base, 153.18 M_{r2} = molecular weight of norphenylephrine as hydrochloride salt, 189.64

Calculate the percentage of phenylephrine related compound C as free base in the portion of Phenylephrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of phenylephrine related compound C from the *Sample solution* r_S = peak response of phenylephrine related compound C from the *Standard solution* C_S = concentration of USP Phenylephrine Related Compound C RS in the *Standard solution* (mg/mL) C_U = concentration of Phenylephrine Hydrochloride in the *Sample solution* (mg/mL) M_{r1} = molecular weight of phenylephrine related compound C as free base, 165.19 M_{r2} = molecular weight of phenylephrine related compound C as hydrochloride salt, 201.65

Calculate the percentage of phenylephrine related compound D in the portion of Phenylephrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of phenylephrine related compound D from the *Sample solution* r_S = peak response of phenylephrine related compound D from the *Standard solution* C_S = concentration of USP Phenylephrine Related Compound D RS in the *Standard solution* (mg/mL) C_U = concentration of Phenylephrine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of phenylephrine related compound E as free base in the portion of Phenylephrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of phenylephrine related compound E from the *Sample solution* r_S = peak response of phenylephrine related compound E from the *Standard solution* C_S = concentration of USP Phenylephrine Related Compound E RS in the *Standard solution* (mg/mL) C_U = concentration of Phenylephrine Hydrochloride in the *Sample solution* (mg/mL) M_{r1} = molecular weight of phenylephrine related compound E as free base, 255.31 M_{r2} = molecular weight of phenylephrine related compound E as hydrochloride salt, 291.77

Calculate the percentage of any individual unspecified impurity in the portion of Phenylephrine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of each unspecified impurity from the *Sample solution* r_S = peak response of phenylephrine from the *Standard solution*

- C_s = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)
 C_u = concentration of Phenylephrine Hydrochloride in the *Sample solution* (mg/mL)
Acceptance criteria: See Table 2. Disregard any peaks below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Norphenylephrine	0.9	0.10
Phenylephrine	1.0	—
Phenylephrine related compound C	1.3	0.1
Phenylephrine related compound D	3.8	0.10
Phenylephrine related compound E	4.0	0.1
Any individual unspecified impurity	—	0.10
Total impurities	—	0.2

SPECIFIC TESTS• **OPTICAL ROTATION, Specific Rotation (781S)**

Sample solution: 50 mg/mL in water

Acceptance criteria: -43° to -47°

• **LOSS ON DRYING (731)**

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25° , excursions permitted between 15° and 30° .• **USP REFERENCE STANDARDS (11)**

USP Norphenylephrine Hydrochloride RS
 3-(2-Amino-1-hydroxyethyl)phenol hydrochloride.

$C_8H_{11}NO_2 \cdot HCl$ 189.64

USP Phenylephrine Hydrochloride RS

USP Phenylephrine Related Compound C RS
 1-(3-Hydroxyphenyl)-2-(methylamino)ethan-1-one hydrochloride.

$C_9H_{11}NO_2 \cdot HCl$ 201.65

USP Phenylephrine Related Compound D RS
 (R)-3-[2-[Benzyl(methyl)amino]-1-hydroxyethyl]phenol.

$C_{16}H_{19}NO_2$ 257.33

USP Phenylephrine Related Compound E RS
 2-[Benzyl(methyl)amino]-1-(3-hydroxyphenyl)ethan-1-one hydrochloride.

$C_{16}H_{17}NO_2 \cdot HCl$ 291.77

Phenylephrine Hydrochloride Injection

» Phenylephrine Hydrochloride Injection is a sterile solution of Phenylephrine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_9H_{13}NO_2 \cdot HCl$.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards (11)—

USP Endotoxin RS

USP Phenylephrine Hydrochloride RS

Identification—Concentrate or dilute, if necessary, a suitable volume of Injection to a concentration of about 10 mg per mL. Apply 2 μ L of this solution and of a Standard solution of USP Phenylephrine Hydrochloride RS, containing about 10 mg per mL, at points about 2.5 cm from the bottom edge of a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Dry the spots in a current of warm air, and develop the chromatogram in a suitable chromatographic chamber with a mixture of methanol, water, and ammonium hydroxide (72:25:3) until the solvent front has moved about 12 cm. Dry the plate in warm air, and spray it with alcoholic potassium hydroxide TS. Dry at 60° for 15 minutes, and spray the plate with *p*-nitroaniline TS: the reddish orange spot obtained from the test solution corresponds in color, size, and intensity to that obtained from the Standard solution.

Bacterial Endotoxins Test (85)—It contains not more than 25.0 USP Endotoxin Units per mg of phenylephrine hydrochloride.

pH (791): between 3.0 and 6.5.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Mobile phase—Prepare and filter a mixture of methanol and water (1:1) containing 1.1 g of sodium 1-octanesulfonate per liter, adjusted with 3 M phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Dilution solvent—Prepare a mixture of methanol and water (1:1), adjusted with 3 M phosphoric acid to a pH of 3.0.

System suitability solution—Dissolve about 50 mg each of USP Phenylephrine Hydrochloride RS and USP Epinephrine Bitartrate RS in 5 mL of water, dilute with *Dilution solvent* to 25.0 mL, and mix. Further dilute 5.0 mL of the resulting solution with *Dilution solvent* to 25.0 mL, and mix to obtain a solution having a concentration of about 0.4 mg of phenylephrine hydrochloride and 0.4 mg of epinephrine bitartrate per mL.

Standard preparation—Dissolve about 50 mg of USP Phenylephrine Hydrochloride RS, accurately weighed, in 10 mL of water, dilute with *Dilution solvent* to 25.0 mL, and mix. Further dilute 5.0 mL of the resulting solution with *Dilution solvent* to 25.0 mL, and mix to obtain a solution having a known concentration of about 0.4 mg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of phenylephrine hydrochloride, to a 25-mL volumetric flask. Dilute with *Dilution solvent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm \times 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the responses for the major peaks: the resolution, R , between epinephrine and phenylephrine is not less than 1.0. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_9H_{13}NO_2 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(25C/V)(r_u/r_s)$$

in which C is the concentration, in mg per mL, of USP Phenylephrine Hydrochloride RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and r_u and r_s are

the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenylephrine Hydrochloride Nasal Jelly

» Phenylephrine Hydrochloride Nasal Jelly contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_9H_{13}NO_2 \cdot HCl$.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Phenylephrine Hydrochloride RS

Identification—Dissolve a suitable quantity in water to obtain a solution having a concentration of about 60 µg per mL, and centrifuge, if necessary: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Phenylephrine Hydrochloride RS, concomitantly measured.

Minimum fill (755): meets the requirements.

Assay—

Mobile phase—Prepare a mixture of methanol and water (1:1) containing 1.1 g of sodium 1-octanesulfonate per liter, adjust with phosphoric acid to a pH of 3.0, filter, and degas. Make adjustments to the methanol and water ratio, if necessary (see *System Suitability* under *Chromatography* (621)).

Dilution solvent—Prepare a mixture of methanol and water (1:1), and adjust with phosphoric acid to a pH of 3.0.

Standard preparation—Dissolve an accurately weighed quantity of USP Phenylephrine Hydrochloride RS in *Dilution solvent* to obtain a Stock standard solution having a known concentration of about 2 mg per mL. Dilute an accurately measured volume of this solution with *Dilution solvent* to obtain the *Standard preparation* having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer an accurately weighed amount of Nasal Jelly, equivalent to about 10 mg of phenylephrine hydrochloride, to a 100-mL volumetric flask. Dilute with *Dilution solvent* to volume, and mix.

Resolution solution—Transfer 5.0 mL of Stock standard solution to a 100-mL volumetric flask, add 10 mg of USP Epinephrine Bitartrate RS, dilute with *Dilution solvent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*: the resolution, R , is not less than 1.5, and the tailing factor for the phenylephrine peak is not more than 2.0. Chromatograph replicate injections of the *Standard preparation*: the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_9H_{13}NO_2 \cdot HCl$ in the portion of Nasal Jelly taken by the formula:

$$100C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Phenylephrine Hydrochloride RS in the *Standard preparation*, and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenylephrine Hydrochloride Nasal Solution

» Phenylephrine Hydrochloride Nasal Solution contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_9H_{13}NO_2 \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Phenylephrine Hydrochloride RS

Identification—It responds to the *Identification* test under *Phenylephrine Hydrochloride Injection*.

Assay—

Mobile phase, *Dilution solvent*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Phenylephrine Hydrochloride Nasal Jelly*.

Assay preparation—Transfer an accurately measured volume of Nasal Solution, equivalent to about 10 mg of phenylephrine hydrochloride, to a 100-mL volumetric flask. Dilute with *Dilution solvent* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_9H_{13}NO_2 \cdot HCl$ in each mL of the Nasal Solution taken by the formula:

$$100(C / V)(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Phenylephrine Hydrochloride RS in the *Standard preparation*, V is the volume, in mL, of Nasal Solution taken, and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenylephrine Hydrochloride Ophthalmic Solution

» Phenylephrine Hydrochloride Ophthalmic Solution is a sterile, aqueous solution of Phenylephrine Hydrochloride. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_9H_{13}NO_2 \cdot HCl$. It may contain a suitable antimicrobial agent and buffer and may contain suitable antioxidants.

Packaging and storage—Preserve in tight, light-resistant containers of not more than 15-mL size.

USP Reference standards (11)—

USP Phenylephrine Hydrochloride RS

Identification—It responds to the *Identification* test under *Phenylephrine Hydrochloride Injection*.

Sterility Tests (71): meets the requirements.

pH (791): between 4.0 and 7.5 for buffered Ophthalmic Solution; between 3.0 and 4.5 for unbuffered Ophthalmic Solution.

Assay—

Mobile phase, *Dilution solvent*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Phenylephrine Hydrochloride Nasal Jelly*.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of phenylephrine hydrochloride, to a 100-mL volumetric flask. Dilute with *Dilution solvent* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_9H_{13}NO_2 \cdot HCl$ in each mL of Ophthalmic Solution taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Phenylephrine Hydrochloride RS in the *Standard preparation*, *V* is the volume, in mL, of Ophthalmic Solution taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenylephrine Hydrochloride Tablets

DEFINITION

Phenylephrine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$).

IDENTIFICATION

- **A.** The UV absorption spectra of the phenylephrine peak of the *Sample solution* and that of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.
- **B.** The retention time of the phenylephrine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

It is suggested to use plastic vials for analysis.

Buffer: 3.45 g/L of monobasic ammonium phosphate in water. Adjust with 10% phosphoric acid or 10% ammonium hydroxide solution to a pH of 4.5 ± 0.10 , if necessary.

Solution A: Dilute 10 mL of glacial acetic acid with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (35:65)

Diluent: Methanol and *Solution A* (30:70)

Standard solution: 0.1 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*

Sample solution: Nominally 0.1 mg/mL of phenylephrine hydrochloride prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask, add 50% of the final volume of *Solution A*, and stir vigorously for NLT 30 min. Add 30% of the final volume of methanol and stir for NLT an additional 90 min. To ensure that particles do not collect above the solvent level, periodically rinse the particulate into the solution with *Solution A*. Allow the resulting solution to cool to room temperature and dilute with *Solution A* to volume. Pass a portion through a suitable filter of 0.45- μ m pore size. Discard the first 2–3 mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm. For *Identification A*, use a diode-array detector in the range of 200–350 nm.

Column: 4.6-mm \times 10-cm; 5- μ m packing L9

Flow rate: 2.0 mL/min

Injection volume: 25 μ L

Run time: NLT 1.75 times the retention time of phenylephrine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.5–3.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phenylephrine from the *Sample solution*

r_S = peak response of phenylephrine from the *Standard solution*

C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phenylephrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

It is suggested to use plastic vials for analysis.

Medium: Simulated gastric fluid without pepsin; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Buffer, Mobile phase, and System suitability: Proceed as directed in the *Assay*.

Standard solution: ($L/900$) mg/mL of USP Phenylephrine Hydrochloride RS in *Medium*, where *L* is the label claim of phenylephrine hydrochloride in mg/Tablet

Sample solution: Pass a portion of the solution under test through a suitable filter of 10–20- μ m pore size.

Chromatographic system: Proceed as directed in the *Assay*, except for the following.

Injection volume: 100 μ L

Run time: NLT 1.5 times the retention time of phenylephrine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response of phenylephrine from the *Sample solution*

r_S = peak response of phenylephrine from the *Standard solution*

C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 75% (*Q*) of the labeled amount of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES

Change to read:

ORGANIC IMPURITIES

Solution A: Trifluoroacetic acid and water (1:1000)

Solution B: Trifluoroacetic acid and acetonitrile (1:1000)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	96.5	3.5
3	96.5	3.5
44	62.0	38.0
45	96.5	3.5
50	96.5	3.5

Diluent: Phosphoric acid and water (0.5:1000)

System suitability solution: 0.0002 mg/mL of USP Phenylephrine Related Compound F RS, 0.0002 mg/mL of USP Phenylephrine Related Compound G RS, and 0.2 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*

Standard solution: 0.002 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent*

Sensitivity solution: 0.0002 mg/mL of USP Phenylephrine Hydrochloride RS in *Diluent* from the *Standard solution*

Sample solution: Nominally 0.2 mg/mL of phenylephrine hydrochloride prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask, add about 50% of the final volume of *Diluent*, and shake for NLT 1 h. Dilute with *Diluent* to volume. Pass a portion through a suitable filter of 0.45- μ m pore size. Discard the first 2 mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm \times 15-cm; 3.5- μ m packing L1

Column temperature: 35.0°

Flow rate: 1.2 mL/min

Injection volume: 50 μ L

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.0 between phenylephrine related compound G and phenylephrine, *System suitability solution*

Relative standard deviation: NMT 6.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual degradation product from the *Sample solution*

r_S = peak response of phenylephrine from the *Standard solution*

C_S = concentration of USP Phenylephrine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phenylephrine hydrochloride in the *Sample solution* (mg/mL)

F = relative response factor of each individual degradation product (see Table 2)

Acceptance criteria: See Table 2. Disregard any peaks below 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Isoquinoline 4,6-diol analog ^a	0.67	0.70	1.0
Phenylephrine related compound F ^b	0.88	1.0	0.3
Phenylephrine related compound G ^c	0.94	1.0	0.3
Phenylephrine	1.00	1.0	—
Phenylephrine (Phenylephrine related compound C) ^d	1.29	1.8	0.5
3-Hydroxybenzaldehyde	3.00	3.1	0.3
Phenylephrine isoquinolinone analog ^e	4.19	8.0	0.3
Any unspecified degradation product	—	1.0	0.3
Total degradation products	—	—	2.4

^a 2-Methyl-1,2,3,4-tetrahydroisoquinoline-4,6-diol.

^b 2-Methyl-1,2,3,4-tetrahydroisoquinoline-4,8-diol.

^c (R)-N-(2-Hydroxy-2-(3-hydroxyphenyl)ethyl)-N-methylglycine.

^d 1-(3-Hydroxyphenyl)-2-(methylamino)ethan-1-one.

^e 1-(3-Hydroxybenzoyl)-2-methylisoquinolin-6(2H)-one.

• (Postponed indefinitely) • (RB 1-May-2016)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at 20°–25°.

Change to read:

• USP REFERENCE STANDARDS (11)

USP Phenylephrine Hydrochloride RS

USP Phenylephrine Related Compound F RS

2-Methyl-1,2,3,4-tetrahydroisoquinoline-4,8-diol.

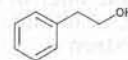
$C_{10}H_{13}NO_2$ 179.22 • (Postponed indefinitely) • (RB 1-May-2016)

USP Phenylephrine Related Compound G RS

(R)-N-(2-Hydroxy-2-(3-hydroxyphenyl)ethyl)-N-methylglycine.

$C_{11}H_{15}NO_4$ 225.24 • (Postponed indefinitely) • (RB 1-May-2016)

Phenylethyl Alcohol



$C_8H_{10}O$

Benzeneethanol;

Phenethyl alcohol [60-12-8].

122.16

IDENTIFICATION• **A. INFRARED ABSORPTION** (197F)**IMPURITIES**• **RESIDUE ON IGNITION** (281)

Sample: 10 mL

Analysis: Evaporate the *Sample* in a suitable crucible, and ignite to constant weight.

Acceptance criteria: NMT 0.005%

SPECIFIC TESTS• **SPECIFIC GRAVITY** (841): 1.017–1.020• **REFRACTIVE INDEX** (831): 1.531–1.534 at 20°• **CHLORINATED COMPOUNDS**

Sample: 2 drops

Analysis: Wind a 1.5- × 5-cm strip of 20-mesh copper gauze around the end of a copper wire. Heat the gauze in the nonluminous flame of a Bunsen burner until it glows without coloring the flame green. Permit the gauze to cool, and heat several times until a good coat of oxide has formed. Apply the *Sample* to the cooled gauze with a medicine dropper, ignite, and permit it to burn freely in the air. Again cool the gauze, add a second *Sample*, and burn as before. Continue this process until a total of 6 drops has been added and ignited, and then hold the gauze in the outer edge of the Bunsen flame, adjusted to a height of about 4 cm.

Acceptance criteria: No transient green color or other color is imparted to the flame.

• **ALDEHYDE**

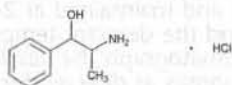
Sample: 5 mL

Analysis: Shake the *Sample* with 5 mL of 1 N sodium hydroxide, and allow to stand for 1 h.

Acceptance criteria: No yellow color appears in the organic (top) layer.

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a cool, dry place.• **USP REFERENCE STANDARDS** (11)

USP Phenylethyl Alcohol RS

Phenylpropanolamine Hydrochloride $C_9H_{13}NO \cdot HCl$

187.67

Benzenemethanol, α -(1-aminoethyl)-, hydrochloride, (*R*,S**)-, (\pm);(\pm)-Norephedrine hydrochloride [154-41-6].**DEFINITION**

Phenylpropanolamine Hydrochloride contains NLT 98.0% and NMT 101.0% of phenylpropanolamine hydrochloride ($C_9H_{13}NO \cdot HCl$), calculated on the dried basis.

IDENTIFICATION• **A. INFRARED ABSORPTION** (197K)• **B. ULTRAVIOLET ABSORPTION** (197U)Solution: 500 μ g/mL

Medium: Water

Analytical wavelength: 256 nm

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

• **C. MELTING RANGE OR TEMPERATURE** (741)

Sample: Dissolve 1 g in 10 mL of water, add 10 mL of saturated sodium carbonate solution, and mix. Separate the precipitate by vacuum filtration, using a sintered-

glass filter of medium pore size. Wash with three 5-mL portions of ice-cold water. Dry the crystals at 80° for 1 h.

Acceptance criteria: The *Sample* melts between 101° and 104°.**ASSAY**• **PROCEDURE**

Sample: 500 mg

Analysis: Dissolve the *Sample* in 50 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS and 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 18.77 mg of phenylpropanolamine hydrochloride ($C_9H_{13}NO \cdot HCl$).

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES• **RESIDUE ON IGNITION** (281): NMT 0.1%**Delete the following:**• **HEAVY METALS, Method I** (231)

Sample solution: Dissolve 1 g in 5 mL of water, add 1 mL of 1 N acetic acid, and dilute with water to 25 mL.

Acceptance criteria: NMT 0.002% (Official 1-Jan-2018)

• **LIMIT OF CATHINONE HYDROCHLORIDE**

Diluent: Dilute hydrochloric acid (1 in 120)

Standard solution: 100 μ g/mL of USP Cathinone Hydrochloride RS in *Diluent*Sample solution: 100 mg/mL of Phenylpropanolamine Hydrochloride in *Diluent***Instrumental conditions**

Mode: UV

Analytical wavelength: Maximum absorbance at about 285 nm

Cell: 1 cm

Blank: *Diluent*

Analysis: Concomitantly determine the absorbances of the *Sample solution* and the *Standard solution*, using the *Blank*.

Acceptance criteria: The absorbance of the *Sample solution* is NMT than that of the *Standard solution* (NMT 0.10%).

• **LIMIT OF AMPHETAMINE HYDROCHLORIDE**

Mobile phase: Acetonitrile, phosphoric acid, triethylamine, and water (50:8:5:950)

System suitability solution: 5 μ g/mL each of USP Phenylpropanolamine Hydrochloride RS and USP Dextroamphetamine Sulfate RS in water

Sample stock solution: 250 mg/mL of Phenylpropanolamine Hydrochloride in water. Sonicate if necessary.

Sample solution: 100 mg/mL of Phenylpropanolamine Hydrochloride in water from *Sample stock solution*Amphetamine standard stock solution: 2.5 μ g/mL of USP Dextroamphetamine Sulfate RS in water

Standard solution: 1 μ g/mL of USP Dextroamphetamine Sulfate RS and 100 mg/mL of Phenylpropanolamine Hydrochloride in water from *Amphetamine standard stock solution* and *Sample stock solution*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 206 nm

Column: 4.6-mm × 25-cm; 5- μ m base-deactivated packing L1

Flow rate: 1 mL/min

Injection volume: 5 μ L**System suitability**

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for phenylpropanolamine and amphetamine are 1.0 and 2.1, respectively.]

Suitability requirements

Resolution: NLT 15 between phenylpropanolamine and amphetamine, *System suitability solution*

Column efficiency: NLT 10,000 theoretical plates, *System suitability solution*

Relative standard deviation: NMT 3.0% for amphetamine, *Standard solution*

Analysis

Samples: *Sample solution* and *Standard solution*

Record the chromatograms, and measure the responses at the locus of the amphetamine peak.

Calculate the percentage of amphetamine hydrochloride in the portion of Phenylpropanolamine Hydrochloride taken:

$$\text{Result} = [r_u / (r_s - r_u)] \times (C_s / C_u) \times (M_{r1} / M_{r2}) \times 0.2$$

r_u = amphetamine peak response from the *Sample solution*

r_s = amphetamine peak response from the *Standard solution*

C_s = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (μg/mL)

C_u = concentration of Phenylpropanolamine Hydrochloride in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of amphetamine hydrochloride, 171.67

M_{r2} = molecular weight of amphetamine sulfate, 368.49

Acceptance criteria: NMT 0.001%

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE, Class I (741):** 191°–196°

• **PH (791)**

Sample solution: A solution (3 in 100)

Acceptance criteria: 4.2–5.5

• **LOSS ON DRYING (731)**

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **LABELING:** Label it to indicate that it is for veterinary use only.

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP Cathinone Hydrochloride RS

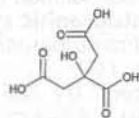
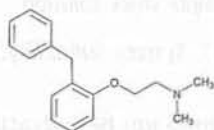
α-Aminopropiophenone hydrochloride.

$C_9H_{11}NO \cdot HCl$ 185.65

USP Dextroamphetamine Sulfate RS

USP Phenylpropanolamine Hydrochloride RS

Phenyltoloxamine Citrate



$C_{17}H_{21}NO \cdot C_6H_8O_7$ 447.48

N,N-Dimethyl-2-(α-phenyl-*o*-tolyl-oxy)ethylamine, citrate (1:1) salt.

2-(2-Dimethylaminoethoxy)diphenylmethane, citrate (1:1) salt

Phenyltoloxamine dihydrogen citrate [1176-08-5].

» Phenyltoloxamine Citrate contains not less than 99.0 percent and not more than 101.0 percent of $C_{17}H_{21}NO \cdot C_6H_8O_7$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers. Store at room temperature.

USP Reference standards (11)—

USP Phenyltoloxamine Citrate RS

USP Phenyltoloxamine Related Compound A RS

2-(2-Benzylphenoxy)ethylmethylamine hydrochloride.

$C_{16}H_{19}NO \cdot HCl$ 277.79

Identification, Infrared Absorption (197K).

Melting range, Class 1a (741): between 137° and 143°.

pH (791): between 3.2 and 4.2, in a solution (1 in 100).

Loss on drying (731)—Dry it in vacuum at 80° for 3 hours; it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.1%.

Delete the following:

• **Heavy metals, Method I (231):** 20 μg per g. • (Official 1-Jan-2018)

Related compounds—

Resolution solution—In a separatory funnel dissolve about 10 mg each of USP Phenyltoloxamine Citrate RS and USP Phenyltoloxamine Related Compound A RS, accurately weighed, in 50 mL of water. Add 5 mL of ammonium hydroxide, and extract with three 10-mL portions of methylene chloride. Combine the extracts, dry the solution over anhydrous sodium sulfate, and gently evaporate to dryness. Dissolve the residue in 20 mL of methylene chloride.

Test solution—In a separatory funnel dissolve about 400 mg of Phenyltoloxamine Citrate, accurately weighed, in 50 mL of water. Proceed as directed for *Resolution solution*, beginning with "Add 5 mL of ammonium hydroxide."

Chromatographic system (see Chromatography (621))—The gas chromatograph is equipped with a split injection system, a flame-ionization detector, and a 0.32-mm × 25-m column coated with a 0.45-μm film of phase G27. The carrier gas is helium, flowing at a rate of about 29 cm per second, with a split flow rate of about 25 mL per minute. The column temperature is programmed as follows. Initially the temperature of the column is equilibrated at 190° for 3 minutes, then the temperature is increased at a rate of 4° per minute to 240°, and maintained at 240° for 8 minutes. The injection port and the detector temperatures are maintained at 280°. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, R , between phenyltoloxamine and phenyltoloxamine related compound A is not less than 1.5.

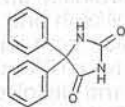
Procedure—Inject a volume (about 1 μL) of the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Phenyltoloxamine Citrate taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response of each impurity; and r_s is the sum of the responses of all the peaks, excluding the solvent peaks: not more than 0.2% of phenyltoloxamine related compound A; not more than 0.1% of any other individual impurity; and not more than 1.0% of total impurities is found.

Assay—Dissolve about 0.5 g of Phenyltoloxamine Citrate, accurately weighed, in 80 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 44.75 mg of $C_{17}H_{21}NO \cdot C_6H_8O_7$.

Phenytoin

C₁₅H₁₂N₂O₂

252.27

2,4-Imidazolidinedione, 5,5-diphenyl-;
5,5-Diphenylhydantoin [57-41-0].

DEFINITION

Phenytoin contains NLT 98.0% and NMT 102.0% of phenytoin (C₁₅H₁₂N₂O₂), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (17K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Solution A: Prepare a 0.05 M monobasic potassium phosphate solution and adjust with phosphoric acid to a pH of 2.5.

Solution B: Methanol and acetonitrile (60:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
23	60	40
38	42	58
45	30	70
50	30	70
51	60	40
55	60	40

Diluent: *Solution B* and water (1:1)

Standard solution: 0.2 mg/mL of USP Phenytoin RS in *Diluent*. Dissolve with the aid of sonication if necessary.

Sample solution: 0.2 mg/mL of Phenytoin in *Diluent*. Dissolve with the aid of sonication if necessary.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 3-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of phenytoin (C₁₅H₁₂N₂O₂) in the portion of Phenytoin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)

C_u = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-Jan-2018)

ORGANIC IMPURITIES

Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 1 μg/mL of USP Phenytoin RS, 5 μg/mL of USP Phenytoin Related Compound A RS, 9 μg/mL of USP Phenytoin Related Compound B RS, and 1 μg/mL of USP Benzophenone RS in *Diluent*

Sample solution: 1 mg/mL of Phenytoin in *Diluent*

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times are given in *Table 2*.]

Suitability requirements

Signal-to-noise ratio: NLT 10

Relative standard deviation: NMT 5.0% for the phenytoin peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Phenytoin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak area of specified impurity from the *Sample solution*

r_s = peak area of corresponding impurity from the *Standard solution*

C_s = concentration of corresponding impurity in the *Standard solution* (mg/mL)

C_u = concentration of Phenytoin in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Phenytoin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak area for each unspecified impurity

r_s = peak area of phenytoin from the *Standard solution*

C_s = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)

C_u = concentration of Phenytoin in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phenytoin related compound A	0.14	0.5
Phenytoin related compound B	0.53	0.9
Phenytoin	1.0	—
Benzophenone	2.11	0.1
Benzil	2.23	—

* Excluding benzophenone.

Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	0.10
Total impurities ^a	—	0.9

^a Excluding benzophenone.**SPECIFIC TESTS**• **LOSS ON DRYING** (731)

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in tight containers.• **USP REFERENCE STANDARDS** (11)

USP Benzophenone RS

Diphenylmethanone.

C₁₃H₁₀O 182.22

USP Phenytoin RS

USP Phenytoin Related Compound A RS

2,2-Diphenylglycine.

C₁₄H₁₃NO₂ 227.26

USP Phenytoin Related Compound B RS

2,2-Diphenyl-2-ureidoacetic acid.

C₁₅H₁₄N₂O₃ 270.28**Phenytoin Oral Suspension****DEFINITION**

Phenytoin Oral Suspension is Phenytoin suspended in a suitable medium. It contains NLT 95.0% and NMT 105.0% of the labeled amount of phenytoin (C₁₅H₁₂N₂O₂).

IDENTIFICATION• **A. INFRARED ABSORPTION** (197K)

Sample: Shake a volume of Oral Suspension equivalent to 100 mg of phenytoin with 50 mL of a mixture of ether and chloroform (1 in 2) in a separator, evaporate the extract to dryness, and dry under vacuum at 105° for 4 h. Weigh 2–4 mg of the residue and 200 mg of potassium bromide in a mortar. Pestle, mix, and grind well, and prepare the potassium bromide pellet.

Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**ASSAY**• **PROCEDURE**

Solution A: Prepare a 0.05 M monobasic potassium phosphate solution, and adjust with phosphoric acid to a pH of 2.5.

Solution B: Methanol and acetonitrile (60:40)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
23	60	40
38	42	58
45	30	70
50	30	70
51	60	40
55	60	40

Diluent: *Solution B* and water (1:1)

Standard solution: 0.2 mg/mL of USP Phenytoin RS in *Diluent*. Dissolve with the aid of sonication, if necessary.

Sample solution: Nominally 0.2 mg/mL of phenytoin prepared as follows. Weigh and transfer a suitable volume of Oral Suspension containing the equivalent of 20 mg of phenytoin to a 100-mL volumetric flask. Add 20 mL of methanol, and dissolve. Dilute with *Diluent* to volume. Dissolve with the aid of sonication, if necessary.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 3-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenytoin (C₁₅H₁₂N₂O₂) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phenytoin in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)

Buffer 1: Dissolve 36.3 g of tris(hydroxymethyl)amino-methane and 60 g of sodium lauryl sulfate in 6 L of water, adjust with hydrochloric acid to a pH of 7.5, and degas.

Medium: *Buffer 1*; 900 mL

Apparatus 2: 35 rpm

Time: 60 min

Buffer 2: 2.76 g/L of monobasic sodium phosphate in water

Mobile phase: Methanol, acetonitrile, and *Buffer 2* (27:23:50). Adjust with phosphoric acid to a pH of 3.0.

Standard solution: 0.14 mg/mL of USP Phenytoin RS prepared as follows. Transfer a suitable amount of USP Phenytoin RS to a suitable volumetric flask. Dissolve in 3% of the flask volume of methanol. Dilute with *Medium* to volume.

Sample solution: Shake the sample suspension well (100 shakes). Determine the density, d (g/mL), of Oral Suspension using appropriate means. Using a 5-mL syringe, collect approximately 5 mL of Oral Suspension, and record the weight. With the paddles lowered, gently empty the contents of each syringe into the bottom of each vessel containing *Medium*. Start rotating the paddles. Reweigh each syringe, and determine the weight (g) of Oral Suspension delivered into each vessel. At the end of 60 min, remove 4 mL from each vessel, and pass through a nylon filter of 0.45-μm pore size, presaturated with *Medium*. [NOTE—Dilute with *Medium* if necessary to a concentration that is similar to the *Standard solution*.]

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitabilitySample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for phenytoin

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times (d/W) \times (1/L) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Phenytoin RS in the *Standard solution* V = volume of *Medium*, 900 mL D = dilution factor (necessary only if the *Sample solution* requires dilution) d = density of Oral Suspension (g/mL) W = weight of Oral Suspension delivered (g) L = label claim of Oral Suspension (mg/mL)Tolerances: NLT 80% (Q) of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$) is dissolved.**• UNIFORMITY OF DOSAGE UNITS (905)**

For single-unit containers

Acceptance criteria: Meets the requirements

• DELIVERABLE VOLUME (698)

For multiple-unit containers

Acceptance criteria: Meets the requirements

IMPURITIES**• ORGANIC IMPURITIES**

Mobile phase, Diluent, and Chromatographic system:

Proceed as directed in the *Assay*.Standard solution: 1 µg/mL of USP Phenytoin RS, 9 µg/mL of USP Phenytoin Related Compound A RS, and 9 µg/mL of USP Phenytoin Related Compound B RS in *Diluent*Sample solution: 1 mg/mL of Oral Suspension in *Diluent*

System suitability

Sample: *Standard solution*

Suitability requirements

Signal-to-noise ratio: NLT 10

Relative standard deviation: NMT 5.0% for the phenytoin peak

AnalysisSamples: *Standard solution* and *Sample solution*[NOTE—The relative retention times are given in *Table 2*.]

Calculate the percentage of phenytoin related compound A and phenytoin related compound B in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak area of each specified impurity from the *Sample solution* r_S = peak area of each specified impurity from the *Standard solution* C_S = concentration of each specified impurity in the *Standard solution* (mg/mL) C_U = nominal concentration of phenytoin in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak area for each unspecified impurity r_S = peak area of phenytoin from the *Standard solution* C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL) C_U = nominal concentration of phenytoin in the *Sample solution* (mg/mL)Acceptance criteria: See *Table 2*. Disregard any impurity less than 0.05%.**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phenytoin related compound A	0.14	0.9
Phenytoin related compound B	0.53	0.9
Phenytoin	1.0	—
Any individual unspecified degradation product	—	0.10
Total impurities	—	0.9

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature. Protect from freezing and light.
- LABELING:** The label bears a statement that the patient must use an accurately calibrated measuring device with multiple-dose containers.
- USP REFERENCE STANDARDS (11)**
 - USP Phenytoin RS
 - USP Phenytoin Related Compound A RS
 - 2,2-Diphenylglycine.
 $C_{14}H_{13}NO_2$ 227.26
 - USP Phenytoin Related Compound B RS
 - 2,2-Diphenyl-2-ureidoacetic acid.
 $C_{15}H_{14}N_2O_3$ 270.28

Phenytoin Chewable Tablets**DEFINITION**Phenytoin Chewable Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$).**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Solution A: Triethylamine and water (1:99)

Mobile phase: Methanol, acetonitrile, water, *Solution A*, and acetic acid (270:230:500:5:1)Standard solution: 0.5 mg/mL of USP Phenytoin RS in *Mobile phase*Sample solution: Nominally 0.5 mg/mL of phenytoin from NLT 20 finely powdered Tablets in *Mobile phase*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitabilitySample: *Standard solution***Suitability requirements**

Column efficiency: NLT 6500 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of phenytoin (C₁₅H₁₂N₂O₂) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL) C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS**• DISSOLUTION (711)**

Buffer: Dissolve 60.5 g of tris(hydroxymethyl)amino-methane in 6 L of water. Dilute with water to 10 L, and adjust with phosphoric acid to a pH of 9.0 ± 0.05. Dissolve 100 g of sodium lauryl sulfate in 6 L of the prepared buffer, transfer this solution to the remaining buffer solution, and mix.

Medium: *Buffer*, 900 mL**Apparatus 2:** 100 rpm**Time:** 120 min**Solution A, Mobile phase, and Chromatographic system:** Proceed as directed in the *Assay*.**Standard stock solution:** 3.0 mg/mL of USP Phenytoin RS in methanol

Standard solution: 0.012 mg/mL of USP Phenytoin RS prepared as follows. Transfer a suitable volume of *Standard stock solution* to a suitable volumetric flask, and dilute with *Medium* to volume to obtain an intermediate concentration of 0.06 mg/mL of USP Phenytoin RS. Transfer a suitable volume of the resulting solution to a suitable volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Withdraw a portion of the solution under test and filter, discarding the first 3 mL of the filtrate. Pipet 10.0 mL of this solution into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of phenytoin (C₁₅H₁₂N₂O₂) dissolved:

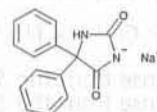
$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL) V = volume of *Medium*, 900 mL L = label claim (mg/Tablet)Tolerances: NLT 70% (Q) of the labeled amount of phenytoin (C₁₅H₁₂N₂O₂) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature. Protect from moisture.
- **LABELING:** Label the Tablets to indicate that they are to be chewed.
- **USP REFERENCE STANDARDS (11)**
USP Phenytoin RS

Phenytoin Sodium

C₁₅H₁₁N₂NaO₂ 274.25
2,4-Imidazolidinedione, 5,5-diphenyl-, monosodium salt;
5,5-Diphenylhydantoin sodium salt [630-93-3].

DEFINITION

Phenytoin Sodium contains NLT 98.0% and NMT 102.0% of phenytoin sodium (C₁₅H₁₁N₂NaO₂), calculated on the dried basis.

IDENTIFICATION**• A. INFRARED ABSORPTION (197K)****• B. IDENTIFICATION TESTS—GENERAL, Sodium (191)**

Solution A: Dissolve 2.7 g of methoxyphenylacetic acid in 6 mL of tetramethylammonium hydroxide solution, and add 20 mL of dehydrated alcohol.

Solution B: 158 mg/mL of ammonium carbonate in water

Sample solution: Ignite 1 g, and cool. Add 2 mL of water to the residue, and neutralize the solution with hydrochloric acid. Filter, and dilute the filtrate with water to 4 mL.

Analysis: To 0.1 mL of the *Sample solution* add 1.5 mL of *Solution A*, and cool in ice water for 30 min. A voluminous, white, crystalline precipitate is formed. Place in water at 20°, and stir for 5 min.

Acceptance criteria: The precipitate does not disappear. Add 1 mL of ammonia TS. The precipitate dissolves completely. Add 1 mL of *Solution B*. No precipitate is formed.

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY**• PROCEDURE**

Buffer: 0.05 M monobasic ammonium phosphate buffer, adjusted with phosphoric acid to a pH of 2.5

Mobile phase: Acetonitrile, methanol, and *Buffer* (35:20:45)

System suitability solution: 0.1 mg/mL of USP Phenytoin RS and 0.15 mg/mL of benzoin in *Mobile phase*

Standard solution: 0.05 mg/mL of USP Phenytoin RS in *Mobile phase*

Sample solution: 0.05 mg/mL of Phenytoin Sodium in *Mobile phase*

Chromatographic system(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for phenytoin and benzoin are 1.0 and 1.3, respectively.]

Suitability requirements**Resolution:** NLT 1.5 between phenytoin and benzoin, *System suitability solution***Tailing factor:** NMT 1.5, *Standard solution***Relative standard deviation:** NMT 1.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) in the portion of Phenytoin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL) C_U = concentration of Phenytoin Sodium in the *Sample solution* (mg/mL) M_{r1} = molecular weight of phenytoin sodium, 274.25 M_{r2} = molecular weight of phenytoin, 252.27**Acceptance criteria:** 98.0%–102.0% on the dried basis**IMPURITIES****Delete the following:**

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm • (Official 1-Jan-2018)

• **ORGANIC IMPURITIES****Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.**Standard solution:** 0.5 μg/mL of benzophenone, 1 μg/mL of USP Phenytoin RS, 9 μg/mL of USP Phenytoin Related Compound A RS, and 9 μg/mL of USP Phenytoin Related Compound B RS in *Mobile phase***Sample solution:** 1 mg/mL of Phenytoin Sodium in *Mobile phase***System suitability****Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for phenytoin and benzoin are 1.0 and 1.3, respectively.]

Suitability requirements**Resolution:** NLT 1.5 between phenytoin and benzoin, *System suitability solution***Relative standard deviation:** NMT 5.0% for each compound, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of phenytoin related compound A, phenytoin related compound B, and benzophenone in the portion of Phenytoin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response of phenytoin related compound A, phenytoin related compound B, or benzophenone from the *Sample solution* r_S = peak response of phenytoin related compound A, phenytoin related compound B, or benzophenone from the *Standard solution* C_S = concentration of the corresponding analyte in the *Standard solution* (μg/mL) C_U = concentration of Phenytoin Sodium in the *Sample solution* (μg/mL)

Calculate the percentage of any unspecified impurity in the portion of Phenytoin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of each impurity from the *Sample solution* r_S = peak response of phenytoin from the *Standard solution* C_S = concentration of USP Phenytoin RS in the *Standard solution* (μg/mL) C_U = concentration of Phenytoin Sodium in the *Sample solution* (μg/mL) M_{r1} = molecular weight of phenytoin sodium, 274.25 M_{r2} = molecular weight of phenytoin, 252.27**Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (% w/w)
Phenytoin related compound A	0.5	0.5
Phenytoin related compound B	0.6	0.9
Phenytoin	1.0	—
Benzophenone	2.9	0.1
Any individual unspecified impurity	—	0.10
Total impurities ^a	—	0.9

^a Excluding benzophenone.**SPECIFIC TESTS**• **LOSS ON DRYING** (731)**Analysis:** Dry at 105° for 4 h.**Acceptance criteria:** NMT 2.5%**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Phenytoin RS

USP Phenytoin Related Compound A RS

Diphenylglycine.

 $C_{14}H_{13}NO_2$ 227.26

USP Phenytoin Related Compound B RS

Diphenylhydantoic acid.

 $C_{15}H_{14}N_2O_3$ 270.29

USP Phenytoin Sodium RS

Extended Phenytoin Sodium Capsules**DEFINITION**Extended Phenytoin Sodium Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$).**IDENTIFICATION**• **A. INFRARED ABSORPTION—GENERAL** (197)**Sample:** 300 mg of phenytoin sodium from the contents of Capsules in 50 mL of water in a separator. Add 10 mL of 3 N hydrochloric acid, and extract with three

successive portions, measuring 100, 60, and 30 mL, respectively, of ether and chloroform (1:2). Evaporate the combined extracts, and dry the residue of phenytoin at 105° for 4 h.

Acceptance criteria: The spectrum of the *Sample* corresponds to that of a similarly prepared USP Phenytoin RS.

- **B.** The retention time of the major peak of the *Sample* solution corresponds to that of the *Standard* solution, as obtained in the *Assay*.

ASSAY

PROCEDURE

Buffer: 0.05 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Methanol and *Buffer* (55:45)

Standard solution: 0.6 mg/mL of USP Phenytoin RS in *Mobile phase*. [NOTE—Dissolve the required quantity of phenytoin in a small amount of methanol before diluting with *Mobile phase*.]

Sample stock solution: Transfer the contents of 10 Capsules to a 250-mL volumetric flask. Add 150 mL of methanol, and sonicate for 20 min. Cool to room temperature, and dilute with methanol to volume.

Sample solution: Nominally 0.6 mg/mL of phenytoin from the *Sample stock solution* in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 229 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of phenytoin from the *Sample solution*

r_S = peak response of phenytoin from the *Standard solution*

C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of phenytoin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of phenytoin sodium, 274.25

M_{r2} = molecular weight of phenytoin, 252.27

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Test 1

Medium: Water; 900 mL

Apparatus 1: 50 rpm

Times: 30, 60, and 120 min

Mobile phase: Methanol and water (70:30)

Standard solution: Dissolve USP Phenytoin RS in methanol, and dilute with water to obtain a concentration similar to that of the *Sample solution*.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 229 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3200 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times V \times (100/L)$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Phenytoin RS in the *Standard solution* (mg/mL)

M_{r1} = molecular weight of phenytoin sodium, 274.25

M_{r2} = molecular weight of phenytoin, 252.27

V = volume of *Medium*, 900 mL

L = label claim (mg/Capsule)

Tolerances (for products labeled as 30-mg Capsules)

The percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved is NMT 40% (Q) in 30 min, 56% (Q') in 60 min, and NLT 65% (Q'') in 120 min. The requirements are met if the quantities dissolved from the Capsules tested conform to *Table 1*.

Table 1

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range between $Q - 15\%$ and $Q - 5\%$, is within the range $Q' \pm 10\%$, and is NLT $Q'' + 5\%$ at the stated <i>Times</i> .
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between $Q - 10\%$ and Q , is within the range $Q' \pm 8\%$, and is NLT Q'' ; no unit is outside the range between $Q - 20\%$ and $Q + 10\%$, no unit is outside the range $Q' \pm 18\%$, and no unit is less than $Q'' - 10\%$ at the stated <i>Times</i> .
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between $Q - 10\%$ and Q , is within the range $Q' \pm 8\%$, and is NLT Q'' ; NMT 2 units are outside the range between $Q - 20\%$ and $Q + 10\%$, and no unit is outside the range $Q - 30\%$ and $Q + 20\%$; NMT 2 units are outside the range $Q' \pm 18\%$, and no unit is outside the range $Q' \pm 25\%$; NMT 2 units are less than $Q'' - 10\%$, and no unit is less than $Q'' - 20\%$ at the stated <i>Times</i> .

Tolerances (for products labeled as 100-mg Capsules)

The percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved is NMT 45% (Q) in 30 min, 60% (Q') in 60 min, and NLT 70% (Q'') in 120 min. The requirements are met if the quantities dissolved from the Capsules tested conform to *Table 2*.

Table 2

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range between $Q - 25\%$ and $Q - 5\%$, is equal to $Q' \pm 20\%$, and is NLT $Q'' + 5\%$ at the stated Times.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between $Q - 20\%$ and Q , is within the range $Q' \pm 15\%$, and is NLT Q'' ; no unit is outside the range between $Q - 30\%$ and $Q + 10\%$, no unit is outside the range $Q' \pm 25\%$, and no unit is less than $Q'' - 10\%$ at the stated Times.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between $Q - 20\%$ and Q , is within the range $Q' \pm 15\%$, and is NLT Q'' ; NMT 2 units are outside the range between $Q - 30\%$ and $Q + 10\%$, and no unit is outside the range between $Q - 40\%$ and $Q + 20\%$; NMT 2 units are outside the range $Q' \pm 25\%$, and no unit is outside the range $Q' \pm 35\%$; NMT 2 units are less than $Q'' - 10\%$, and no unit is less than $Q'' - 20\%$ at the stated Times.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Proceed as directed in *Test 1*, except use *Apparatus 1* at 75 rpm and the following *Tolerances*.

Tolerances (for products labeled as 100-mg Capsules)

The percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved is NMT 45% (Q) in 30 min, 65% (Q') in 60 min, and NLT 70% (Q'') in 120 min. The requirements are met if the quantities dissolved from the Capsules tested conform to *Table 3*.

Table 3

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range between $Q - 25\%$ and $Q - 5\%$, is equal to $Q' \pm 20\%$, and is NLT $Q'' + 5\%$ at the stated Times.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between $Q - 25\%$ and $Q - 5\%$, is within the range of $Q' - 20\%$ and $Q' + 10\%$, and is NLT Q'' ; no unit is outside the range between $Q - 30\%$ and $Q + 5\%$, no unit is outside the range $Q' - 25\%$ and $Q' + 20\%$, and no unit is less than $Q'' - 10\%$ at the stated Times.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between $Q - 25\%$ and $Q - 5\%$, is within the range of $Q' - 20\%$ and $Q' + 10\%$, and is NLT Q'' ; NMT 2 units are outside the range between $Q - 30\%$ and $Q + 5\%$; and no unit is outside the range of $Q - 40\%$ and $Q + 15\%$; NMT 2 units are outside the range $Q' - 25\%$ and $Q' + 20\%$, and no unit is outside the range $Q' - 35\%$ and $Q' + 25\%$; NMT 2 units are less than $Q'' - 10\%$; and no unit is less than $Q'' - 20\%$ at the stated Times.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: Water; 900 mL

Apparatus 1: 75 rpm

Times: 30, 60, and 120 min

Determine the amount of phenytoin sodium

($C_{15}H_{11}N_2NaO_2$) dissolved by using the method described in *Test 1*.

Tolerances (for products labeled as 200- and 300-mg Capsules)

The percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved is NMT 30% (Q) in 30 min, 50% (Q') in 60 min, and NLT 60% (Q'') in 120 min. The requirements are met if the quantities dissolved from the Capsules tested conform to *Table 4*.

Table 4

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range between $Q - 20\%$ and $Q + 5\%$, is equal to $Q' - 20\%$ and $Q' + 25\%$, and is NLT $Q'' + 5\%$ at the stated Times.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between $Q - 20\%$ and Q , is within the range of $Q' \pm 20\%$, and is NLT Q'' ; no unit is outside the range between $Q - 25\%$ and $Q + 10\%$, no unit is outside the range $Q' \pm 25\%$, and no unit is less than $Q'' - 10\%$ at the stated Times.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between $Q - 20\%$ and Q , is within the range of $Q' \pm 20\%$, and is NLT Q'' ; NMT 2 units are outside the range between $Q - 25\%$ and $Q + 10\%$, and no unit is outside the range $Q - 25\%$ and $Q + 15\%$; NMT 2 units are outside the range $Q' \pm 25\%$; and no unit is outside the range $Q' \pm 30\%$; NMT 2 units are less than $Q'' - 10\%$; and no unit is less than $Q'' - 20\%$ at the stated Times.

Test 4: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium, Apparatus 1, Times, and Analysis: Proceed as directed for *Test 1*.

Tolerances (for products labeled as 30-mg Capsules):

The percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) dissolved is NMT 40% (Q) in 30 min, 56% (Q') in 60 min, and NLT 65% (Q'') in 120 min. The requirements are met if the quantities dissolved from the Capsules tested conform to *Table 5*.

Table 5

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is within the range between $Q - 10\%$ and Q , is within the range $Q' - 9\%$ and $Q' + 7\%$, and is NLT $Q'' + 5\%$ at the stated Times.
S_2	6	Average of 12 units ($S_1 + S_2$) is within the range between $Q - 8\%$ and $Q + 2\%$, is within the range $Q' - 9\%$ and $Q' + 7\%$, and is NLT Q'' ; no unit is outside the range between $Q - 20\%$ and $Q + 10\%$, no unit is outside the range $Q' - 19\%$ and $Q' + 17\%$, and no unit is less than $Q'' - 10\%$ at the stated Times.
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between $Q - 8\%$ and $Q + 2\%$, is within the range $Q' - 9\%$ and $Q' + 7\%$, and is NLT Q'' ; NMT 2 units are outside the range between $Q - 20\%$ and $Q + 10\%$, and no unit is outside the range $Q - 30\%$ and $Q + 20\%$; NMT 2 units are outside the range $Q' - 19\%$ and $Q' + 17\%$, and no unit is outside the range $Q' - 26\%$ and $Q' + 24\%$; NMT 2 units are less than $Q'' - 10\%$, and no unit is less than $Q'' - 20\%$ at the stated Times.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

IMPURITIES• **ORGANIC IMPURITIES**

Buffer, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 600 µg/mL of USP Phenytoin RS, 3 µg/mL of USP Phenytoin Related Compound A RS, and 3 µg/mL of USP Phenytoin Related Compound B RS in methanol

System suitability

Sample: Standard solution

[NOTE—The relative retention times for phenytoin related compound A, phenytoin related compound B, and phenytoin are 0.38, 0.45, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8 between phenytoin related compound B and phenytoin; NLT 1.5 between phenytoin related compound A and phenytoin related compound B

Tailing factor: NMT 2.0 for the phenytoin peak

Relative standard deviation: NMT 2.0% for phenytoin; NMT 5.0% for phenytoin related compound A or phenytoin related compound B

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each phenytoin related compound in the portion of Capsules taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of phenytoin related compound A or phenytoin related compound B from the Sample solution

r_s = peak response of phenytoin related compound A or phenytoin related compound B from the Standard solution

C_s = concentration of the corresponding analyte in the Standard solution (µg/mL)

C_u = nominal concentration of phenytoin in the Sample solution (µg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of each unspecified degradation product from the Sample solution

r_s = peak response of phenytoin from the Standard solution

C_s = concentration of USP Phenytoin RS in the Standard solution (µg/mL)

C_u = nominal concentration of phenytoin in the Sample solution (µg/mL)

Acceptance criteria: See Table 6.

Table 6

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phenytoin related compound A	0.38	0.5
Phenytoin related compound B	0.45	1.0
Phenytoin	1.0	—
Any individual, unspecified degradation product	—	0.2

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Protect from moisture. Store at controlled room temperature.
- **LABELING:** When more than one Dissolution test is given, the labeling states the Dissolution test used only if Test 1 is not used.
- **USP REFERENCE STANDARDS (11)**
 - USP Phenytoin RS
 - USP Phenytoin Related Compound A RS
 - Diphenylglycine.
 - $C_{14}H_{13}NO_2$ 227.26
 - USP Phenytoin Related Compound B RS
 - Diphenylhydantoic acid.
 - $C_{15}H_{14}N_2O_3$ 270.29

Delete the following:

▲ Prompt Phenytoin Sodium Capsules

» Prompt Phenytoin Sodium Capsules contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{15}H_{11}N_2NaO_2$.

Packaging and storage—Preserve in tight containers.

Labeling—Label the Capsules with the statement "Not for once-a-day dosing," printed immediately under the official name, in a bold and contrasting color and/or enclosed within a box.

USP Reference standards (11)—

USP Phenytoin RS

USP Phenytoin Sodium RS

Identification—

A: The contents of Capsules respond to Identification test A under Phenytoin Sodium.

B: The contents of Capsules respond to the flame test for Sodium (191).

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 1: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{15}H_{11}N_2NaO_2$ dissolved by measuring the UV absorbance at 258 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium* if necessary, in comparison with a *Standard solution* having a known concentration of *USP Phenytoin Sodium RS* in the same *Medium*.

Tolerances—Not less than 85% (Q) of the labeled amount of $C_{15}H_{11}N_2NaO_2$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Procedure for content uniformity—Proceed as directed in the test for *Uniformity of dosage units* under *Extended Phenytoin Sodium Capsules*.

Assay—Proceed with Capsules as directed in the *Assay* under *Extended Phenytoin Sodium Capsules*. Δ_{USP40}

Phenytoin Sodium Injection

DEFINITION

Phenytoin Sodium Injection is a sterile solution of Phenytoin Sodium with Propylene Glycol and Alcohol in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$).

IDENTIFICATION• **A. INFRARED ABSORPTION** (197K)

Sample: Transfer an equivalent of 250 mg of phenytoin sodium from a volume of Injection to a separator containing 25 mL of water. Extract first with 50 mL of ethyl acetate and then with two additional 30-mL portions of ethyl acetate. Wash each extract with two 20-mL portions of sodium acetate solution (10 mg/mL). Evaporate the combined ethyl acetate extracts, and dry the residue of phenytoin at 105° to constant weight.

Acceptance criteria: Residue obtained from the *Sample* meets the requirements.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Mobile phase: Methanol and water (55:45)

Standard solution: 0.23 mg/mL of *USP Phenytoin RS* in *Mobile phase*

Sample solution: Nominally 0.25 mg/mL of phenytoin sodium from a volume of Injection equivalent to 250 mg of phenytoin sodium in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of *USP Phenytoin RS* in the *Standard solution* (mg/mL)

C_U = nominal concentration of phenytoin sodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of phenytoin sodium, 274.25

M_{r2} = molecular weight of phenytoin, 252.27

Acceptance criteria: 95.0%–105.0%

OTHER COMPONENTS• **ALCOHOL AND PROPYLENE GLYCOL CONTENT**

Internal standard solution: 0.02 mL/mL of methanol and 0.04 mL/mL of ethylene glycol in water

Standard stock solution: 0.01 mL/mL of alcohol from *USP Alcohol Determination—Alcohol RS* and 0.04 mL/mL of *USP Propylene Glycol RS* in water

Standard solution: 0.005 mL/mL of alcohol and 0.02 mL/mL of propylene glycol prepared as follows. Pipet 5 mL each of *Standard stock solution* and *Internal standard solution* into a 10-mL volumetric flask.

Sample solution: 0.05 mL/mL of Injection in water prepared as follows. Pipet 5 mL of Injection and 50 mL of the *Internal standard solution* into a 100-mL volumetric flask, and dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m × 0.53-mm (ID) capillary column with a 1-µm coating of G16 phase

Temperatures

Injection port: 240°

Detector: 250°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	4
50	15	230	5

Carrier gas: Hydrogen or helium

Flow rate: 5 mL/min

Injection volume: 0.2 µL

Injection type: Split ratio, 10:1

System suitability

Sample: *Standard solution*

[NOTE—See Table 2 for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between methanol and alcohol; NLT 5.0 between propylene glycol and ethylene glycol

Relative standard deviation: NMT 2.0% for each of the response ratios of alcohol to methanol and propylene glycol to ethylene glycol

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the alcohol content, as a percentage, in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of alcohol to methanol from the *Sample solution*

R_S = peak response ratio of alcohol to methanol from the *Standard solution*

C_S = concentration of alcohol in the *Standard solution* (mL/mL)

C_U = nominal concentration of the Injection in the *Sample solution* (mL/mL)

Calculate the propylene glycol content, as a percentage, in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of propylene glycol to ethylene glycol from the *Sample solution*

R_S = peak response ratio of propylene glycol to ethylene glycol from the *Standard solution*

C_S = concentration of propylene glycol in the *Standard solution* (mL/mL)

C_U = nominal concentration of the Injection in the *Sample solution* (mL/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methanol*	0.27	—
Alcohol	0.32	9.0–11.0
Propylene glycol	0.98	37.0–43.0
Ethylene glycol*	1.0	—

* Internal standard included for peak identification purposes only.

SPECIFIC TESTS

- BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.3 USP Endotoxin Unit/mg of phenytoin sodium.
- pH (791):** 10.0–12.3
- PARTICULATE MATTER IN INJECTIONS (788):** It meets the requirements under small-volume injections.
- OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products (1)*.

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, at controlled room temperature.
- LABELING:** The label states the following: "Do not use the Injection if it is hazy or contains a precipitate."
- USP REFERENCE STANDARDS (11)**
 - USP Alcohol Determination—Alcohol RS
 - USP Endotoxin RS
 - USP Phenytoin RS
 - USP Propylene Glycol RS

Chromic Phosphate P 32 Suspension

» Chromic Phosphate P 32 Suspension is a sterile, aqueous suspension of radioactive chromic phos-

phate P 32 in a 30 percent Dextrose solution suitable for intraperitoneal, intrapleural, or interstitial administration. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{32}P as chromic phosphate expressed in megabecquerels (millicuries) per mL at the time indicated in the labeling. It may contain a preservative or a stabilizer. Other chemical forms of radioactivity do not exceed 5.0 percent of the total radioactivity.

Packaging and storage—Preserve in single-dose or multiple-dose containers.

Labeling—Label it to include the following, in addition to the information specified *Labeling (7)*, *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of ^{32}P as labeled chromic phosphate expressed as total megabecquerels (millicuries) and concentration as megabecquerels (millicuries) per mL at the time of calibration; the expiration date; and the statements, "Caution—Radioactive Material," and "For intracavitary use only." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{32}P is 14.3 days.

USP Reference standards (11)—

USP Endotoxin RS

Radionuclide identification—

A: The beta radiation of the Suspension, measured according to the procedure set forth under *Radioactivity (821)*, shows a mass absorption coefficient within $\pm 5\%$ of the value found for a specimen of a known standard of the same radionuclide when determined under identical counting conditions and geometry.

B: Its beta-ray spectrum is identical to that of a specimen of ^{32}P of known purity showing no distinct photopeaks and no energies greater than 1.710 MeV.

Bacterial Endotoxins Test (85)—It contains not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

pH (791): between 3.0 and 5.0.

Radiochemical purity—Place a measured volume of Suspension, to provide a count rate of about 20,000 counts per minute, about 2.5 cm from one end of a 25-mm \times 300-mm strip of chromatographic paper (see *Chromatography (621)*), and allow to dry. Develop the chromatogram by ascending chromatography, using water as the solvent, and air-dry: the radioactivity in the chromic phosphate is not less than 95.0% of the total radioactivity when measured at the origin.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products (1)*, except that the Suspension may be distributed or dispensed prior to the completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendations on *Container Content*.

Assay for dextrose—

Periodic acid reagent solution—Dissolve 8.5 g of sodium metaperiodate in 80 mL of 1 N sulfuric acid, dilute with water to 100 mL, and mix.

Assay preparation—Decant the supernatant from sterile Suspension into a disposable centrifuge tube, and centrifuge. Pipet 1.0 mL of the clear supernatant into a 25-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Pipet 50 mL of *Periodic acid reagent solution* into a 250-mL conical flask, add 3.0 mL of the *Assay preparation*, swirl, cover the flask, and allow to stand at room temperature for 2 hours. Add, in the order named and with rapid stirring, 50 mL of a saturated solution of sodium bicar-

bonate, 50.0 mL of 0.1 N potassium arsenite VS, 4 mL of potassium iodide solution (1 in 5), and 20 g of sodium bicarbonate. Stir the solution at room temperature for 15 minutes. Titrate with 0.1 N iodine VS, using 3 mL of starch TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N iodine is equivalent to 1.802 mg of dextrose ($C_6H_{12}O_6$). Not less than 27.0% and not more than 33.0% is found.

Change to read:

Assay for radioactivity—Using a suitable counting assembly (CN 1-May-2017), determine the radioactivity, in MBq (mCi) per mL, of Sterile Suspension by use of a calibrated system as directed under *Radioactivity* (821).

Sodium Phosphate P 32 Solution

Phosphoric- ^{32}P acid, disodium salt.
Dibasic sodium phosphate- ^{32}P [7635-46-3].

» Sodium Phosphate P 32 Solution is a solution suitable for either oral or intravenous administration, containing radioactive phosphorus (^{32}P) processed in the form of Dibasic Sodium Phosphate from the neutron bombardment of elemental sulfur. Nonradioactive Dibasic Sodium Phosphate may be added during the processing.

Sodium Phosphate P 32 Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{32}P as phosphate expressed in megabecquerels (microcuries or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity are absent.

Packaging and storage—Preserve in single-dose or multiple-dose containers that previously have been treated to prevent adsorption.

Labeling—Label it to include the following: the time and date of calibration; the amount of ^{32}P as phosphate expressed in total megabecquerels (microcuries or millicuries) and in megabecquerels (microcuries or in millicuries) per mL at the time of calibration; the name and quantity of any added preservative or stabilizer; a statement of the intended use, whether oral or intravenous; a statement of whether the contents are intended for diagnostic or therapeutic use; the expiration date; and the statements "Caution—Radioactive Material," and "Not for intracavitary use." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of ^{32}P is 14.3 days.

USP Reference standards (11)—
USP Endotoxin RS

Radionuclide identification—

A: The beta radiation of the Solution, measured according to the procedure set forth under *Radioactivity* (821), shows a mass absorption coefficient within $\pm 5\%$ of the value found for a specimen of a known standard of the same radionuclide when determined under identical counting conditions and geometry.

B: Its beta-ray and/or bremsstrahlung spectrum is identical to that of a specimen of ^{32}P of known purity showing no distinct photopeaks and no energies greater than 1.710 MeV.

Bacterial Endotoxins Test (85)—It contains not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

pH (791): between 5.0 and 6.0.

Radiochemical purity—Place a measured volume, appropriately diluted with phosphoric acid solution (1 in 20) such that it provides a count rate of about 20,000 counts per minute, about 45 mm from the end of a 25- \times 300-mm strip of chromatographic paper (see *Chromatography* (621)), and allow to dry. Develop the chromatogram by descending chromatography, using a mixture of tertiary butyl alcohol, water, and formic acid (40:20:5). Allow to dry, and determine the position of the phosphoric acid by spraying the paper with a solution prepared by dissolving 5 g of ammonium molybdate in 100 mL of water and pouring, with constant stirring, into a mixture of 12 mL of nitric acid and 24 mL of water. Determine the position of the radioactivity distribution by scanning with a collimated radiation detector. The radioactivity appears in one band only, corresponding in R_f value to the phosphoric acid.

Other requirements—Solution intended for intravenous use meets the requirements under *Injections and Implanted Drug Products* (1), except that the Solution may be distributed or dispensed prior to completion of the test for *Sterility*, the latter test being started on the day of final manufacture, and except that it is not subject to the recommendation on *Container Content*.

Change to read:

Assay for radioactivity—Using a suitable counting assembly (CN 1-May-2017), determine the radioactivity, in MBq (mCi) per mL, of Solution by use of a calibrated system as directed under *Radioactivity* (821)).

Physostigmine Salicylate

$C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$ 413.47
Pyrrolo[2,3-b]indol-5-ol, 1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethyl-, methylcarbamate (ester), (3a*S*-*cis*-), mono(2-hydroxybenzoate);
Physostigmine monosalicylate [57-64-7].

DEFINITION

Physostigmine Salicylate contains NLT 97.0% and NMT 102.0% of physostigmine salicylate ($C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$), calculated on the dried basis.

IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** (181)
Analysis: Use 1 g of sodium bicarbonate instead of 2 mL of 1 N sodium hydroxide.
Acceptance criteria: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL, Salicylate** (191): Meets the requirements

ASSAY

• PROCEDURE

Sample: 250 mg of Physostigmine Salicylate

Analysis: Dissolve the *Sample* in 25 mL of chloroform, and add 25 mL of glacial acetic acid. Titrate with 0.02 N perchloric acid in dioxane VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.02 N perchloric acid is equivalent to 8.270 mg of physostigmine salicylate ($C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$).

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

• RESIDUE ON IGNITION (281)

Sample: 100 mg

Acceptance criteria: Negligible

• SULFATE

Sample solution: Precipitate the salicylic acid from 10 mL of a cold, saturated solution of Physostigmine Salicylate with a slight excess of 3 N hydrochloric acid, and filter.

Analysis: Add 5 drops of barium chloride TS to the Sample solution.

Acceptance criteria: No turbidity is produced immediately.

SPECIFIC TESTS

• OPTICAL ROTATION, Specific Rotation (781S)

Sample solution: 10 mg/mL in water

Acceptance criteria: -91° to -94°

• LOSS ON DRYING (731)

Analysis: Dry over silica gel for 24 h.

Acceptance criteria: NMT 1.0%

• READILY CARBONIZABLE SUBSTANCES TEST (271)

Sample solution: Dissolve 100 mg in 5 mL of sulfuric acid.

Acceptance criteria: At the end of 5 min, the Sample solution has no more color than Matching Fluid I.

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

• USP REFERENCE STANDARDS (11)

USP Physostigmine Salicylate RS

Physostigmine Salicylate Injection

DEFINITION

Physostigmine Salicylate Injection is a sterile solution of Physostigmine Salicylate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of physostigmine salicylate ($C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$). It may contain an antimicrobial agent and an antioxidant.

[NOTE—Do not use the Injection if it is more than slightly discolored.]

IDENTIFICATION

• A. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181)

Analysis: Use 1 g of sodium bicarbonate instead of 2 mL of 1 N sodium hydroxide.

Acceptance criteria: Meets the requirements

• B. IDENTIFICATION TESTS—GENERAL, Salicylate (191): Meets the requirements

ASSAY

• PROCEDURE

Buffer: 3.85 g/L of ammonium acetate in water. Adjust, if necessary, with glacial acetic acid or ammonium hydroxide to a pH of 6 ± 0.1 .

Mobile phase: Acetonitrile and Buffer (50:50)

Solution A: 100 μ L of USP Benzyl Alcohol RS and 1 μ L of benzaldehyde in 400 mL of acetonitrile

Standard solution: 0.03 mg/mL of USP Physostigmine Salicylate RS in Solution A

Sample solution: Nominally 0.03 mg/mL from a suitable volume of Injection containing NLT 3 mg of physostigmine salicylate diluted with acetonitrile

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 μ L

System suitability

Samples: Solution A and Standard solution

[NOTE—If the peaks due to benzyl alcohol and benzaldehyde co-elute when Solution A is injected, the Standard solution will exhibit only two peaks instead of three. In a suitable system, benzyl alcohol and benzaldehyde elute before physostigmine.]

Suitability requirements

Resolution: NLT 2.0 between the physostigmine peak and the adjacent peak (benzyl alcohol or benzaldehyde, or the combination of these), Standard solution

Column efficiency: NLT 1200 theoretical plates from the analyte peak, Standard solution

Relative standard deviation: NMT 2.0%, Standard solution

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of physostigmine salicylate ($C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$) in the portion of Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the Sample solution

r_s = peak response from the Standard solution

C_s = concentration of USP Physostigmine Salicylate RS in the Standard solution (mg/mL)

C_u = nominal concentration of physostigmine salicylate in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• BACTERIAL ENDOTOXINS TEST (85): NMT 83.4 USP Endotoxin Units/mg of physostigmine salicylate

• PH (791): 3.5–5.0

• OTHER REQUIREMENTS: It meets the requirements in Injections and Implanted Drug Products (1).

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in single-dose containers, preferably of Type I glass, protected from light.

• USP REFERENCE STANDARDS (11)

USP Benzyl Alcohol RS

USP Endotoxin RS

USP Physostigmine Salicylate RS

Physostigmine Salicylate Ophthalmic Solution

DEFINITION

Physostigmine Salicylate Ophthalmic Solution is a sterile, aqueous solution of physostigmine salicylate. It contains NLT 90.0% and NMT 110.0% of the labeled amount of physostigmine salicylate ($C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$). It may contain suitable antimicrobial agents, buffers, and stabilizers and suitable additives to increase its viscosity.

IDENTIFICATION

• A. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181)

Analysis: Use 1 g of sodium bicarbonate instead of 2 mL of 1 N sodium hydroxide.

Acceptance criteria: Meets the requirements

• B. IDENTIFICATION TESTS—GENERAL, Salicylate (191): Meets the requirements

ASSAY• **PROCEDURE**

Buffer: 3.85 g/L of ammonium acetate in water adjusted, if necessary, with glacial acetic acid or ammonium hydroxide to a pH of 6 ± 0.1

Mobile phase: Acetonitrile and Buffer (50:50)

Standard solution: 0.03 mg/mL of USP Physostigmine Salicylate RS in acetonitrile

Sample solution: Nominally 0.03 mg/mL of physostigmine salicylate from a volume of Ophthalmic Solution containing NLT 3 mg of physostigmine salicylate diluted with acetonitrile

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm \times 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 μ L

System suitability

Sample: Standard solution

Suitability requirements

Column efficiency: NLT 1200 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of physostigmine salicylate ($C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Physostigmine Salicylate RS in the Standard solution (mg/mL)

C_U = nominal concentration of physostigmine salicylate in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

• **STERILITY TESTS (71):** Meets the requirements

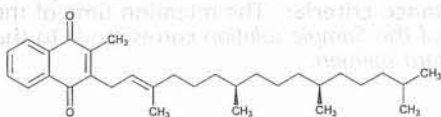
• **PH (791):** 2.0–4.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP Physostigmine Salicylate RS

Phytonadione

$C_{31}H_{46}O_2$ 450.70

1,4-Naphthalenedione, 2-methyl-3-(3,7,11,15-tetramethyl-2-hexadecenyl)-, [*R*-(*R**,*R**(*E*))]—; Phylloquinone [84-80-0].

DEFINITION

Phytonadione is a mixture of *E*- and *Z*-isomers containing NLT 97.0% and NMT 103.0% of phytonadione isomers ($C_{31}H_{46}O_2$). It contains NMT 21.0% of the *Z*-isomer.

IDENTIFICATION

• **A. INFRARED ABSORPTION (197F)**

• **B. ULTRAVIOLET ABSORPTION (197U)**

Analytical wavelength: 248 nm

Sample solution: 10 μ g/mL in *n*-hexane

Acceptance criteria: Meets the requirements. Absorptivities do not differ by more than 3.0%.

ASSAY• **PROCEDURE**

[NOTE—Protect solutions containing phytonadione from exposure to light.]

Mobile phase: *n*-Hexane and *n*-amyl alcohol (2000:1.5)

Internal standard solution: 2.5 mg/mL of cholesteryl benzoate in Mobile phase

Standard solution: Prepare a 96- μ g/mL solution of USP Phytonadione RS in Mobile phase. Pipet 10 mL of this solution and 7 mL of Internal standard solution into a 25-mL volumetric flask, and dilute with Mobile phase to volume.

Sample solution: Prepare as directed for the Standard solution, using Phytonadione instead of USP Phytonadione RS.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; packing L3

Flow rate: 1 mL/min

Injection size: 50 μ L

System suitability

Sample: Standard solution

[NOTE—The relative retention times for the internal standard, (*Z*)-phytonadione, and (*E*)-phytonadione are 0.7, 0.9, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between (*Z*)-phytonadione and (*E*)-phytonadione

Relative standard deviation: NMT 2.0% for the ratios of the sum of peak areas of (*Z*)-phytonadione and (*E*)-phytonadione to the peak area of the internal standard

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of phytonadione ($C_{31}H_{46}O_2$) in the portion of Phytonadione taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = internal standard ratio (sum of peak areas of (*Z*)-phytonadione and (*E*)-phytonadione/peak area of the internal standard) from the Sample solution

R_S = internal standard ratio (sum of peak areas of (*Z*)-phytonadione and (*E*)-phytonadione/peak area of the internal standard) from the Standard solution

C_S = concentration of USP Phytonadione RS in the Standard solution (μ g/mL)

C_U = concentration of Phytonadione in the Sample solution (μ g/mL)

Acceptance criteria: 97.0%–103.0%

OTHER COMPONENTS• **Z-ISOMER CONTENT**

[NOTE—Protect solutions containing phytonadione from exposure to light.]

Mobile phase, Internal standard solution, Sample solution, Chromatographic system, and Analysis: Proceed as directed in the Assay, except calculate the percentage of *Z*-isomer in the portion of Phytonadione taken:

$$\text{Result} = [r_Z/(r_Z + r_E)] \times 100$$

r_z = peak area of the (Z)-phytonadione isomer from the *Sample solution*

r_E = peak area of the (E)-phytonadione isomer from the *Sample solution*

Acceptance criteria: NMT 21.0%

IMPURITIES

• LIMIT OF MENADIONE

Sample: 20 mg of Phytonadione

Analysis: Mix the *Sample* with 0.5 mL of a mixture of 6 N ammonium hydroxide and alcohol (1:1). Add 1 drop of ethyl cyanoacetate, and shake gently.

Acceptance criteria: No purple or blue color is produced.

SPECIFIC TESTS

• **REFRACTIVE INDEX (831):** 1.523–1.526

• **REACTION:** A 50-mg/mL solution of Phytonadione in dehydrated alcohol is neutral to litmus.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**
USP Phytonadione RS

Phytonadione Injectable Emulsion

» Phytonadione Injectable Emulsion is a sterile, aqueous dispersion of Phytonadione. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{31}H_{46}O_2$. It contains suitable solubilizing and/or dispersing agents.

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards (11)—

USP Endotoxin RS

USP Phytonadione RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test (85)—It contains not more than 14.0 USP Endotoxin Units per mg of phytonadione.

pH (791): between 3.5 and 7.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products (1)*.

Assay—[NOTE—Use low-actinic glassware throughout this assay, and otherwise protect the solutions from exposure to light.]

Mobile phase—Prepare a suitable degassed mixture of dehydrated alcohol and water (95:5).

Standard preparation—Dissolve an accurately weighed quantity of USP Phytonadione RS in *Mobile phase* to obtain a solution having a known concentration of about 1 mg per mL. Pipet 1 mL of this solution into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.1 mg per mL.

Assay preparation 1 (containing 10 mg or more of phytonadione per mL)—Pipet a volume of Injectable Emulsion, equivalent to 10 mg of phytonadione, into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and

mix. Pipet 1 mL of this solution into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation 2 (containing less than 10 mg of phytonadione per mL)—Pipet a volume of Injectable Emulsion, equivalent to 1 mg of phytonadione, into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 0.7 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the appropriate *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak response for the major peak. Calculate the quantity, in mg, of $C_{31}H_{46}O_2$ in each mL of the Injectable Emulsion taken by the formula:

$$D(C/V)(r_U / r_S)$$

in which *D* is 100 if the Injectable Emulsion contains 10 mg or more of phytonadione per mL, or 10 if the Injectable Emulsion contains less than 10 mg of phytonadione per mL; *C* is the concentration, in mg per mL, of USP Phytonadione RS in the *Standard preparation*; *V* is the volume, in mL, of Injectable Emulsion taken; and r_U and r_S are the peak responses of phytonadione obtained from the appropriate *Assay preparation* and the *Standard preparation*, respectively.

Phytonadione Tablets

DEFINITION

Phytonadione Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of phytonadione ($C_{31}H_{46}O_2$).

IDENTIFICATION

• A. ULTRAVIOLET ABSORPTION

Standard solution: 0.01 mg/mL of USP Phytonadione RS in dehydrated alcohol

Sample solution: A portion of finely powdered Tablets, equivalent to 0.01 mg/mL of phytonadione in dehydrated alcohol. Shake vigorously, and filter. Use the filtrate.

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution* concomitantly measured.

• B. HPLC IDENTIFICATION TEST

Analysis: Proceed as directed in the *Assay*.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• PROCEDURE

[NOTE—Use low-actinic glassware throughout the *Assay*, and otherwise protect the solutions from light.]

Mobile phase: Dehydrated alcohol and water (95:5)

Standard solution: 0.10 mg/mL of USP Phytonadione RS in dehydrated alcohol

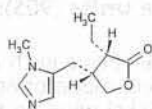
Sample solution: 0.1 mg/mL of phytonadione in dehydrated alcohol prepared as follows. Mix a portion of finely powdered Tablets (NLT 20) with dehydrated alcohol to obtain a nominal concentration of 0.4 mg/mL of phytonadione. Shake by mechanical means for 15 min, dilute with dehydrated alcohol to 0.10 mg/mL, and filter.

Chromatographic system(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4-mm × 30-cm; packing L1**Flow rate:** 1.5 mL/min**Injection size:** 10 µL**System suitability****Sample:** *Standard solution* (three replicate injections)**Suitability requirements****Column efficiency:** NLT 915 theoretical plates, determined from the analyte peak**Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%

[NOTE—Z- and E-isomers coelute in this chromatographic system.]

Analysis**Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of phytonadione (C₃₁H₄₆O₂) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

 r_u = peak area from the *Sample solution* r_s = peak area from the *Standard solution* C_s = concentration of USP Phytonadione RS in the *Standard solution* (mg/mL) C_u = nominal concentration of phytonadione in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**• **DISINTEGRATION** (701)**Time:** 30 min• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.• **USP REFERENCE STANDARDS** (11)
USP Phytonadione RS**Pilocarpine**C₁₁H₁₆N₂O₂ 208.26

2(3H)-Furanone, 3-ethylidihydro-4-[(1-methyl-1H-imidazol-5-yl)methyl]-, (3S-cis)-.

Pilocarpine [92-13-7].

» Pilocarpine contains not less than 95.0 percent and not more than 100.5 percent of pilocarpine (C₁₁H₁₆N₂O₂), calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers, in a cold place.

USP Reference standards (11)—

USP Pilocarpine RS

USP Pilocarpine Nitrate RS

Identification—**A:** *Infrared Absorption* (197F).**B:** *Ultraviolet Absorption* (197U)—**Solution:** 20 µg per mL.**Medium:** water.**Specific rotation** (781S): between +102° and +107°.**Test solution:** 20 mg per mL, in pH 6.0 phosphate buffer.**Refractive index** (831): between 1.5170 and 1.5210 at 25°, determined in a liquid specimen. If crystals are present, first warm to about 40°.**Water Determination, Method I** (921): not more than 0.5%.**Chloride—**

Standard chloride solution—Transfer 165 mg of sodium chloride to a 100-mL volumetric flask, and dissolve in and dilute with water to volume. Transfer 25.0 mL of this solution to a 1000-mL volumetric flask, and dilute with water to volume. This solution contains 25 µg of chloride per mL.

Test solution—Transfer about 1.0 g of Pilocarpine, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume.

Procedure—Transfer 5.0 mL of the *Test solution* to a test tube, add 0.6 mL of diluted nitric acid and 0.3 mL of silver nitrate TS: any opalescence produced is not greater than that produced by an identically treated solution containing 5.0 mL of the *Standard chloride solution* (0.25%).

Sulfate—

Standard sulfate solution—Dissolve 148 mg of anhydrous sodium sulfate in water, and dilute with water to 100 mL. Dilute 10.0 mL of this solution with water to 1000 mL. This solution contains 10 µg of sulfate per mL.

Procedure—To about 1 g of Pilocarpine in a test tube add 1 mL of 6 N hydrochloric acid and 4 mL of water, and mix. For the control, transfer 4.0 mL of *Standard sulfate solution* to a test tube, add 1 mL of 6 N hydrochloric acid, and mix. Adjust both solutions with pH indicator paper by the dropwise addition of 3 N hydrochloric acid or 6 N ammonium hydroxide, if necessary, to a pH of between 2 and 3. Add water to maintain the same volume in the control and test specimen tubes. To each tube add 1 mL of barium chloride TS, and mix: any turbidity produced in the specimen tube after 10 minutes' standing is not greater than that produced in the control (0.004%).

Limit of nitrate—

Standard preparation—Prepare a solution of USP Pilocarpine Nitrate RS to contain 43 µg per mL. This solution contains the equivalent of 10 µg of nitrate ion per mL.

Test preparation—Prepare a solution of Pilocarpine to contain 200 µg per mL.

Procedure—Transfer 0.5-mL portions of the *Test preparation* and of the *Standard preparation*, respectively, to separate test tubes, and to each tube add 1 drop of a 1 in 100 solution of sulfanilic acid in 5 N acetic acid and 1 drop of a 3 in 1000 solution of N-(1-naphthyl)ethylenediamine dihydrochloride in 5 N acetic acid. Adjust the *Standard preparation* and the *Test preparation* with pH indicator paper by the dropwise addition of 3 N hydrochloric acid or 1 N ammonium hydroxide, if necessary, to a pH of between 2 and 3. To each solution add a few granules of acid-washed, nitrate-free zinc. Heat the test tubes in a water bath at a temperature of about 32°. Allow 5 minutes for the development of a pink color: any pink color observed in the *Test preparation* is not greater than that observed in the *Standard preparation* (0.005%).

Related compounds—

Buffer solution, Mobile phase, System suitability preparation, and Chromatographic system—Proceed as directed in the Assay.

Standard solution—Prepare a solution in water of isopilocarpine nitrate to contain 1.5 µg per mL.

Test preparation—Prepare as directed for Assay preparation in the Assay.

Procedure—Separately inject equal volumes (about 40 µL) of the *Standard solution* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the percentage of isopilocarpine in the portion of Pilocarpine taken by the formula:

$$(208.26 / 271.27)50(C / W)(r_U / r_S)$$

in which 208.26 and 271.27 are the molecular weights of pilocarpine and isopilocarpine nitrate, respectively, *C* is the concentration, in µg per mL, of isopilocarpine nitrate in the *Standard solution*, *W* is the weight, in mg, of Pilocarpine taken, and *r_U* and *r_S* are the peak responses due to isopilocarpine in the *Test preparation* and the *Standard solution*, respectively; not more than 2% of isopilocarpine is found. Calculate the percentage of all other impurities from the chromatogram of the *Test preparation* taken by the formula:

$$(208.26 / 271.27)50(C / W)(r_i / r_S)$$

in which *r_i* is the peak response due to the impurity; no one impurity corresponding to one of the four peaks in the *System suitability preparation* exceeds 3%; no other individual impurity exceeds 0.5%. The sum total of all impurities, including isopilocarpine, is not more than 5.0%.

Assay—

Buffer solution—Transfer 13.5 mL of phosphoric acid to a 1-liter beaker containing 700 mL of water. Add 3 mL of triethylamine, and dilute with water to 1000 mL. Adjust with 20% sodium hydroxide to a pH of 3.0.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (98:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—Do not store this *Mobile phase* for more than 2 days.]

Standard preparation—Prepare a solution in water having an accurately known concentration of about 40 µg of USP Pilocarpine Nitrate RS per mL. [NOTE—Use this solution within 24 hours of its preparation.]

Assay preparation—Transfer an accurately weighed quantity of about 15 mg of Pilocarpine to a 500-mL volumetric flask. Dilute with water to volume, and mix. [NOTE—Use this solution within 24 hours of its preparation.]

System suitability preparation—Transfer accurately weighed quantities of about 30 mg each of pilocarpine hydrochloride and isopilocarpine nitrate to a 50-mL volumetric flask, and dilute with water to volume. Transfer 25 mL of this solution to a suitable flask, add 5 mL of 1 N sodium hydroxide, and reflux for 1 hour. Cool, and adjust the solution with 0.25 M phosphoric acid to a pH of 7.0. Quantitatively transfer this solution to a 50-mL volumetric flask, dilute with water to volume, and mix. Dilute the remaining original solution with water to volume, and mix. Add 1 mL each of the refluxed and unrefluxed solutions to a 10-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 12.5-cm column that contains 3-µm packing L1. The flow rate is about 1.0 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*; the relative standard deviation is not more than 2.0%. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*; four peaks are observed; the resolution, *R*, between two adjacent peaks is not less than 1.2, the column efficiency determined for the pilo-

carpine peak is not less than 1500 theoretical plates, and the tailing factor, *T*, for the pilocarpine peak is not greater than 1.5. The relative retention times for the major peaks are about 0.67 for isopilocarpine, 0.76 for pilocarpine, 0.85 for pilocarpic acid, and 1.0 for isopilocarpic acid.

Procedure—Separately inject equal volumes (about 40 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in mg, of pilocarpine (C₁₁H₁₆N₂O₂) in the portion of Pilocarpine taken by the formula:

$$(208.26 / 271.27)500C(r_U / r_S)$$

in which 208.26 and 271.27 are the molecular weights of pilocarpine and pilocarpine nitrate, respectively, *C* is the concentration, in mg per mL, of USP Pilocarpine Nitrate RS in the *Standard preparation*, and *r_U* and *r_S* are the peak responses for pilocarpine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pilocarpine Ocular System

» Pilocarpine Ocular System contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of pilocarpine (C₁₁H₁₆N₂O₂). It is sterile.

Packaging and storage—Preserve in single-dose containers in a cold place.

USP Reference standards (11)—

USP Pilocarpine RS

USP Pilocarpine Hydrochloride RS

USP Pilocarpine Nitrate RS

Identification—Cut around the inside margin of the Ocular System, then discard the ring encircling the Ocular System, extract the remaining portion with 0.5 mL of methanol in a small capped vial, shaking vigorously for 1 to 2 minutes. Evaporate the methanol extract on a sodium chloride plate forming a thin film: the IR absorption spectrum of the film exhibits maxima only at the same wavelengths as that of a similar preparation of USP Pilocarpine RS.

Sterility Tests (71): meets the requirements.

Uniformity of dosage units (905): meets the requirements for capsules.

Drug release pattern—Place each of the Ocular Systems in suitable porous holders made of an inert material, and suspend each from a nickel wire. To the upper end of the wire attach a tag identifying the specimen. Put each assembly into a test tube containing 27.0 mL of saline TS so that the system lies at the bottom of the tube and the identifying tag extends from the open top of the tube. Put the tubes into a horizontally reciprocating shaker in which the temperature is maintained at 37 ± 0.5°. Agitate the tubes with a horizontal amplitude of about 4 cm and a frequency of about 35 cycles per minute. At 7, 24, 48, 72, 96, and 168 hours, remove the assemblies from their tubes, and each time replace them in similar tubes containing 27.0 mL of fresh saline TS. Determine the amount of pilocarpine in solution in each tube, after adjusting the volume to 27.0 mL to make up for any evaporative losses, by measuring the UV absorbance in 1-cm cells at the wavelength of maximum absorbance at about 215 nm, with a suitable spectrophotometer, against saline TS as the blank. Concomitantly measure the absorbance of a Standard solution of USP Pilocarpine Hydrochloride RS having a known concentration of

about 20 µg in each mL of saline TS. Calculate the quantity, in µg, of $C_{11}H_{16}N_2O_2$ in each solution taken by the formula:

$$(208.26 / 244.72)(A_U / A_S)27C$$

in which 208.26 and 244.72 are the molecular weights of pilocarpine and pilocarpine hydrochloride, respectively; A_U and A_S are the absorbances of the test solution and the Standard solution, respectively; and C is the concentration, in µg per mL, of USP Pilocarpine Hydrochloride RS in the Standard solution. Calculate the amount of pilocarpine released in 168 hours by adding the pilocarpine content of each set of tubes collected over 168 hours.

Tolerances—The amount of $C_{11}H_{16}N_2O_2$ from each Ocular System released during the total 0 to 168 hours tested conforms to *Acceptance Table 1* under *Drug Release* (724). The drug release range for this time period is not less than 80.0% and not more than 120.0% of the labeled release pattern.

Assay—

Buffer solution, Mobile phase, Standard preparation, System suitability preparation, and Chromatographic system—Proceed as directed in the Assay under *Pilocarpine*.

Assay preparation—Select not fewer than 10 Ocular Systems. Cut each System into 4 pieces, transfer quantitatively to a 500-mL volumetric flask, and rinse all cutting utensils with 20 to 30 mL of methanol into the flask. Make additional rinses of the utensils with about 250 mL of *Mobile phase*, and collect all the rinses in the flasks. Allow the flasks to stand for 30 minutes, sonicate for about 15 minutes, dilute with water to volume, and mix. Transfer an aliquot of the supernatant, equivalent to 6 mg of pilocarpine to a 200-mL volumetric flask, dilute with water to volume, mix, and filter.

Procedure—Proceed as directed for *Procedure* in the Assay under *Pilocarpine*. Calculate the quantity, in mg, of pilocarpine in each Ocular System taken by the formula:

$$(208.26 / 271.27)(10 / V)(C / N)(r_U / r_S)$$

in which 208.26 and 271.27 are the molecular weights of pilocarpine and pilocarpine nitrate, respectively; V is the volume, in mL, of the supernatant taken (see *Assay preparation*); C is the concentration, in µg per mL, of USP Pilocarpine Nitrate RS in the *Standard preparation*; N is the number of Ocular Systems taken; and r_U and r_S are the peak responses for pilocarpine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pilocarpine Hydrochloride

$C_{11}H_{16}N_2O_2 \cdot HCl$ 244.72
2(3*H*)-Furanone, 3-ethylidihydro-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-, monohydrochloride, (3*S*-*cis*)-;
Pilocarpine monohydrochloride [54-71-7].

DEFINITION

Pilocarpine Hydrochloride contains NLT 98.0% and NMT 102.0% of $C_{11}H_{16}N_2O_2 \cdot HCl$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements
Sample solution: 50 mg/mL

ASSAY

• PROCEDURE

Buffer: 4.4 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.5 ± 0.1.

Mobile phase: Acetonitrile, methanol, and *Buffer* (2:35:63)

Standard solution: 0.5 mg/mL of USP Pilocarpine Hydrochloride RS in water. [NOTE—Sonicate if necessary.]

System suitability solution: Transfer a known quantity of USP Pilocarpine Hydrochloride RS in a suitable volumetric flask, and add water, equivalent to 10% of the volume of the flask, to dissolve. [NOTE—Sonicate as needed.] Add 0.1 N sodium hydroxide, equivalent to 10% of the volume of the flask, quench immediately with the same volume of 0.1 N hydrochloric acid, and mix. Dilute with water to volume. [NOTE—The initial concentration of USP Pilocarpine Hydrochloride RS is 0.5 mg/mL. Isopilocarpine is formed in the *System suitability solution* preparation.]

Sample solution: 0.5 mg/mL of Pilocarpine Hydrochloride in water. [NOTE—Sonicate if necessary.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3-µm packing L11

Column temperature: 35°

Flow rate: 1.0 mL/min

Injection size: 10 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between isopilocarpine and pilocarpine, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{11}H_{16}N_2O_2 \cdot HCl$ in the portion of Pilocarpine Hydrochloride taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Pilocarpine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Organic Impurities

• PROCEDURE 1: RELATED COMPOUNDS

Mobile phase, Standard solution, System suitability solution, and Sample solution: Proceed as directed in the Assay.

Sensitivity solution: 0.25 µg/mL of USP Pilocarpine Hydrochloride RS in water from the *Standard solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3-µm packing L11

Column temperature: 35°

Flow rate: 1.0 mL/min

Run time: NLT 5 times the retention time of the pilocarpine peak

Injection size: 10 µL

System suitability

Samples: *Standard solution*, *System suitability solution*, and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.5 between isopilocarpine and pilocarpine, *System suitability solution*

Signal-to-noise ratio: NLT 10 for the pilocarpine peak, *Sensitivity solution*

Relative standard deviation: NMT 2.0% for the pilocarpine peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Pilocarpine Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak area of each individual impurity from the *Sample solution*

r_s = peak area of pilocarpine from the *Standard solution*

C_s = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)

C_u = concentration of Pilocarpine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria

Individual impurities: See *Impurity Table 1*. [NOTE—Disregard any unspecified impurity peaks less than 0.05%.]

Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Isopilocarpine ^a	0.94	1.0
Pilocarpine	1.00	—
Pilocarpic acid ^b	1.15	0.5
Isopilocarpic acid ^c	1.19	0.1
Any unspecified impurity	—	0.1

^a (3*R*,4*R*)-3-Ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]dihydrofuran-2(3*H*)-one.

^b (2*S*,3*R*)-2-Ethyl-4-hydroxy-3-[(1-methyl-1*H*-imidazol-5-yl)methyl]butanoic acid.

^c (2*R*,3*R*)-2-Ethyl-4-hydroxy-3-[(1-methyl-1*H*-imidazol-5-yl)methyl]butanoic acid.

• PROCEDURE 2: OTHER ALKALOIDS

Sample solution: 10 mg/mL in water

Analysis: Divide the *Sample solution* into two portions. To one portion add a few drops of 6 N ammonium hydroxide, and to the other, add a few drops of potassium dichromate TS.

Acceptance criteria: No turbidity is produced in either solution.

SPECIFIC TESTS

- OPTICAL ROTATION, Specific Rotation (781S):** +88.5° to +91.5°

Sample solution: 20 mg/mL, in water

- LOSS ON DRYING (731):** Dry a sample at 105° for 2 h: it loses NMT 3.0% of its weight.

- READILY CARBONIZABLE SUBSTANCES TEST (271)**

Sample solution: 50 mg/mL in sulfuric acid

Acceptance criteria: The solution has no more color than *Matching Fluid B*.

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.

• USP REFERENCE STANDARDS (11)

USP Pilocarpine Hydrochloride RS

Pilocarpine Hydrochloride Ophthalmic Solution

DEFINITION

Pilocarpine Hydrochloride Ophthalmic Solution is a sterile, buffered, aqueous solution of pilocarpine hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2 \cdot HCl$). It may contain suitable antimicrobial agents and stabilizers, and suitable additives to increase its viscosity.

IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Solution A: Ammonium hydroxide in isopropyl alcohol (1 in 50)

Mobile phase: *n*-Hexane and *Solution A* (700:300)

Standard solution: 1.6 mg/mL of USP Pilocarpine Hydrochloride RS in water

Sample solution: Nominally equivalent to 1.6 mg/mL of pilocarpine hydrochloride from a volume of Ophthalmic Solution diluted with methanol

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L3

Flow rate: 2 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for pilocarpine hydrochloride is 16 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for pilocarpine hydrochloride for three replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2 \cdot HCl$) in each mL of Ophthalmic Solution taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **STERILITY TESTS** (71): Meets the requirements
- **PH** (791): 3.5–5.5

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)
USP Pilocarpine Hydrochloride RS

Pilocarpine Hydrochloride Tablets

DEFINITION

Pilocarpine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2 \cdot HCl$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to the major peak of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Solution A: 10 N sodium hydroxide, 85% phosphoric acid, triethylamine, and water (7:6:1:500). Adjust with 10 N sodium hydroxide to a pH of 3.0.

Mobile phase: Methanol and *Solution A* (3:100)

Standard solution: 50 µg/mL of USP Pilocarpine Hydrochloride RS

System suitability solution: Transfer 10 mL of the *Standard solution* to a test tube. Add 100 µL of 2 N sodium hydroxide, mix well, and allow it to stand for 5 min. Add 100 µL of 2 N hydrochloric acid and mix well.

[NOTE—This preparation contains pilocarpine, isopilocarpine, and two unidentified compounds.]

Sample stock solution: Place Tablets, equivalent to 50 mg of pilocarpine hydrochloride, in a 500-mL volumetric flask. Fill the flask to 75% full with water. Stir for at least 30 min or more if necessary, until the tablets are completely disintegrated and the powder is finely dispersed. Dilute with water to volume.

Sample solution: Transfer 5 mL of *Sample stock solution* to a 10-mL volumetric flask, and dilute with water to volume. Pass a suitable amount of solution through a PVDF, 0.45-µm pore size filter, and discard first 5 mL.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for isopilocarpine, pilocarpine, and two unidentified peaks are 0.9, 1.0, 1.2, and 1.5, respectively.]

Suitability requirements

Resolution: NLT 1.2 between isopilocarpine and pilocarpine; NLT 1.2 between pilocarpine and the peak at a relative retention time of 1.2; NLT 1.2 between peaks at relative retention times of 1.2 and 1.5, *System suitability solution*

Column efficiency: NLT 1500 theoretical plates, *Standard solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{11}H_{16}N_2O_2 \cdot HCl$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pilocarpine hydrochloride from the *Sample solution*

r_S = peak response of pilocarpine hydrochloride from the *Standard solution*

C_S = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pilocarpine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 45 min

Buffer solution: 13.5 mL of phosphoric acid and 3.0 mL of triethylamine in 1000 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 3.

Mobile phase: Methanol and *Buffer solution* (3:17)

Standard stock solution: 0.1 mg/mL of USP Pilocarpine Hydrochloride RS in *Medium*

Standard solution: For Tablets labeled to contain 7.5 mg, transfer 15.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with *Medium* to volume. For Tablets labeled to contain 5 mg, transfer 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Medium* to volume.

Sample solution: Pass the solution under test through a suitable, 45-µm pore size polyethylene filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pilocarpine hydrochloride dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = Tablet label claim (mg)

V = volume of *Medium*, 500 mL

Tolerances: NLT 75% (Q) of the labeled amount of pilocarpine hydrochloride is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

Procedure for content uniformity

Mobile phase, Standard solution, System suitability solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Sample solution: Place 1 Tablet in a suitable volumetric flask, fill the flask about 75% full with water, and

vigorously stir for NLT 30 min to ensure complete disintegration. Dilute with water to volume to obtain a final concentration of 0.05 mg/mL of pilocarpine hydrochloride. Pass the solution through a PVDF, 0.45- μ m pore size filter.

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{11}H_{16}N_2O_2 \cdot HCl$ in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- r_u = peak response of pilocarpine hydrochloride from the *Sample solution*
 r_s = peak response of pilocarpine hydrochloride from the *Standard solution*
 C_s = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)
 C_u = nominal concentration of pilocarpine hydrochloride in the *Sample solution* (mg/mL)

IMPURITIES

Organic Impurities

PROCEDURE

Mobile phase, System suitability solution, and System suitability: Proceed as directed in the *Assay*.

Standard solution: 0.5 μ g/mL of USP Pilocarpine Hydrochloride RS

Sample solution: Pass a suitable amount of *Sample stock solution*, prepared as directed in the *Assay*, through a PVDF, 0.45- μ m pore size filter, and use the filtrate for analysis after discarding the first 5 mL.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Flow rate: 1.5 mL/min

Injection size: 100 μ L

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

- r_u = peak response of each impurity from the *Sample solution*
 r_s = peak response of pilocarpine hydrochloride from the *Standard solution*
 C_s = concentration of USP Pilocarpine Hydrochloride RS in the *Standard solution* (mg/mL)
 C_u = nominal concentration of pilocarpine hydrochloride in the *Sample solution* (mg/mL)
 F = relative response factor for each impurity (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 1.2%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Isopilocarpine	0.9	0.79	1.0
Pilocarpine	1.0	1.0	—
Pilocarpic acid	1.2	1.0	0.5
Individual unspecified degradation product	—	1.0	0.2

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
USP Pilocarpine Hydrochloride RS

Pilocarpine Nitrate

$C_{11}H_{16}N_2O_2 \cdot HNO_3$ 271.27

2(3*H*)-Furanone, 3-ethylidihydro-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-, (3*S*-*cis*)-, mononitrate.

Pilocarpine mononitrate [148-72-1].

» Pilocarpine Nitrate contains not less than 98.5 percent and not more than 101.0 percent of $C_{11}H_{16}N_2O_2 \cdot NO_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Pilocarpine Nitrate RS

Identification—

A: *Infrared Absorption* (197K).

B: Mix a solution (1 in 10) with an equal volume of ferrous sulfate TS, and superimpose the mixture upon 5 mL of sulfuric acid contained in a test tube: the zone of contact becomes brown.

Melting range (741): between 171° and 176°, with decomposition, but the range between beginning and end of melting does not exceed 3°.

Specific rotation (781S): between +79.5° and +82.5°.

Test solution: 20 mg per mL, in water.

Loss on drying (731)—Dry it at 105° for 2 hours: it loses not more than 2.0% of its weight.

Readily carbonizable substances (271)—Dissolve 100 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid A*.

Chloride—To 5 mL of a solution (1 in 50), acidified with nitric acid, add a few drops of silver nitrate TS: no opalescence is produced immediately.

Other alkaloids—Dissolve 200 mg in 20 mL of water, and divide the solution into two portions. To one portion add a few drops of 6 N ammonium hydroxide and to the other add a few drops of potassium dichromate TS: no turbidity is produced in either solution.

Assay—Dissolve about 600 mg of Pilocarpine Nitrate, accurately weighed, in 30 mL of glacial acetic acid, warming slightly to effect solution. Cool to room temperature, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 27.13 mg of $C_{11}H_{16}N_2O_2 \cdot NO_3$.

Pilocarpine Nitrate Ophthalmic Solution

» Pilocarpine Nitrate Ophthalmic Solution is a sterile, buffered, aqueous solution of Pilocarpine Nitrate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{11}H_{16}N_2O_2 \cdot HNO_3$. It may contain suitable antimicrobial agents and stabilizers, and suitable additives to increase its viscosity.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Pilocarpine Nitrate RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

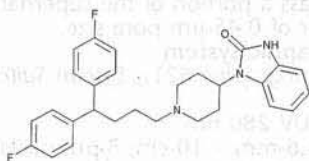
B: It responds to *Identification test B* under *Pilocarpine Nitrate*.

Sterility Tests (71): meets the requirements.

pH (791): between 4.0 and 5.5.

Assay—Proceed with *Ophthalmic Solution* as directed in the *Assay* under *Pilocarpine Hydrochloride Ophthalmic Solution*, except to read pilocarpine nitrate instead of pilocarpine hydrochloride throughout and to calculate the quantity, in mg, of $C_{11}H_{16}N_2O_2 \cdot HNO_3$ in each mL of the *Ophthalmic Solution* taken by the formula given therein.

Pimozide



$C_{28}H_{29}F_2N_3O$ 461.55
2H-Benzimidazol-2-one, 1-[1-[4,4-bis(4-fluorophenyl)butyl]-4-piperidinyl]-1,3-dihydro-;
1-[1-[4,4-Bis(p-fluorophenyl)butyl]-4-piperidyl]-2-benzimidazolinone [2062-78-4].

DEFINITION

Pimozide contains NLT 98.0% and NMT 102.0% of pimozide ($C_{28}H_{29}F_2N_3O$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: 2.5 g/L of ammonium acetate and 8.5 g/L of tetrabutylammonium hydrogen sulfate in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
10	70	30
15	70	30
20	80	20
25	80	20

System suitability solution: 0.05 mg/mL of USP Pimozide RS and 0.02 mg/mL of USP Mebendazole RS in methanol

Standard solution: 1 mg/mL of USP Pimozide RS in methanol

Sample solution: 1 mg/mL of Pimozide in methanol. [NOTE—Sonication may be needed to dissolve the sample.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 10-cm; 3-μm packing L1

Flow rate: 2 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between pimozide and mebendazole, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 0.73% for five injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pimozide ($C_{28}H_{29}F_2N_3O$) in the portion of Pimozide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pimozide from the *Sample solution*

r_S = peak response of pimozide from the *Standard solution*

C_S = concentration of USP Pimozide RS in the *Standard solution* (mg/mL)

C_U = concentration of Pimozide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm • (Official 1-Jan-2018)

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 0.05 mg/mL of USP Pimozide RS in methanol

Sample solution: 10 mg/mL of Pimozide in methanol. [NOTE—Sonication may be needed to dissolve the sample.]

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 5.0 between pimozide and mebendazole, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Pimozide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of pimozide from the *Standard solution*

C_S = concentration of USP Pimozide RS in the *Standard solution* (mg/mL)

C_U = concentration of Pimozide in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Pimozide amine ^a	0.1	0.5
Mebendazole ^b	0.88	—
Desfluoro pimozide ^c	0.9	0.5
o-Pimozide isomer ^d	0.95	0.5
Pimozide	1.0	—
Didehydropimozide ^e	1.1	0.5
Pimozide N-oxide ^f	1.3	0.5
Any individual unspecified impurity	—	0.10
Total impurities	—	0.75

^a 1-(Piperidin-4-yl)benzimidazolin-2-one.

^b Included for system suitability purposes only. Not a process impurity or degradation product.

^c 1-[1-[4-(4-Fluorophenyl)-4-phenylbutyl]piperidin-4-yl]benzimidazolin-2-one.

^d 1-[1-[4-(2-Fluorophenyl)-4-phenylbutyl]piperidin-4-yl]benzimidazolin-2-one.

^e 1-[1-[4,4-Bis(4-fluorophenyl)butyl]-1,2,3,6-tetrahydropyridin-4-yl]benzimidazolin-2-one.

^f 1-[4,4-Bis(4-fluorophenyl)butyl]-4-(2-oxobenzimidazol-1-yl)piperidine 1-oxide.

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry at 105° to a constant weight.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• USP REFERENCE STANDARDS (11)

USP Mebendazole RS

USP Pimozide RS

Pimozide Tablets

DEFINITION

Pimozide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of pimozide ($C_{28}H_{29}F_2N_3O$).

IDENTIFICATION

• A. INFRARED ABSORPTION (197K)

Sample: Grind an appropriate number of Tablets to prepare a 1 mg/mL solution of pimozide in dichloromethane. Shake the solution for 5 min, and pass through a suitable filter. Evaporate the filtrate to dryness under reduced pressure. Add a suitable amount of potassium bromide powder, mix well, and press a small amount into a transparent pellet.

Acceptance criteria: The IR absorption spectrum of the pellet is consistent with that of USP Pimozide RS.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Protect all pimozide solutions from light.

Solution A: 2.5 g/L of ammonium acetate and 8.5 g/L of tetrabutylammonium hydrogen sulfate in water

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
10	70	30
15	70	30
16	80	20
30	80	20

System suitability solution: 0.04 mg/mL of USP Pimozide RS and 0.02 mg/mL of USP Mebendazole RS in methanol

Standard solution: 0.4 mg/mL of USP Pimozide RS in methanol

Sample solution: Nominally 0.4 mg/mL prepared as follows. Transfer a suitable number of powdered Tablets (NLT 20) to a suitable volumetric flask. Add about 70% of the flask volume of methanol, and mechanically shake for 30 min. Dilute with methanol to volume, and mix well with the aid of sonication for 10 min. Centrifuge, and pass a portion of the supernatant through a suitable filter of 0.45-μm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 10-cm; 3-μm packing L1

Flow rate: 2 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*. [NOTE—The relative retention times for mebendazole and pimozide are 0.88 and 1.0 respectively.]

Suitability requirements

Resolution: NLT 5.0 between the pimozide and mebendazole peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% for pimozide, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pimozide ($C_{28}H_{29}F_2N_3O$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of pimozide from the *Sample solution*

r_S = peak response of pimozide from the *Standard solution*

C_S = concentration of USP Pimozide RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pimozide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• DISSOLUTION (711)

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard stock solution: Transfer 27 mg of USP Pimozide RS to a 250-mL volumetric flask containing 1 mL of lactic acid. Heat on a steam bath to dissolve, add 80 mL of hot water, and shake. Cool, dilute with water to volume, and mix.

Standard solution: Dilute the *Standard stock solution* quantitatively with 0.01 N hydrochloric acid to obtain a solution having a known concentration approximately

the same as that of the *Sample solution* (assuming complete dissolution).

Sample solution: Transfer a suitable volume of the solution under test to a suitable container, and centrifuge until clear.

Instrumental conditions

Mode: UV

Analytical wavelength: 277 nm

Cell: 5 cm

Analysis

Samples: *Standard solution* and *Sample solution*, prepared as follows. Pipet a volume of the *Sample solution*, estimated to contain 110 µg of pimozone (assuming complete dissolution), into a suitable container. Pipet an equal volume of the *Standard solution* into a second container. To each container, add 20 mL each of 1 N sodium hydroxide and chloroform. Shake each mixture by mechanical means for 15 min, and centrifuge. Aspirate and discard the aqueous layers, and transfer the chloroform layers to separate clean beakers. Use the chloroform layer for analysis.

Calculate the percentage of the labeled amount of pimozone ($C_{28}H_{29}F_2N_3O$) dissolved from absorbances of the chloroform layers from the *Sample solution* and *Standard solution*:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution* chloroform extract

A_S = absorbance of the *Standard solution* chloroform extract

C_S = concentration of USP Pimozone RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of pimozone ($C_{28}H_{29}F_2N_3O$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS, Content Uniformity (905):** Meet the requirements

IMPURITIES

• ORGANIC IMPURITIES

Protect all pimozone solutions from light.

Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 0.01 mg/mL of USP Pimozone RS in methanol

Sample solution: Nominally 2 mg/mL prepared as follows. Transfer a suitable number of powdered Tablets (NLT 20) to a suitable volumetric flask. Add about 70% of the flask volume of methanol, and mechanically shake for 30 min. Dilute with methanol to volume, and mix well with the aid of sonication for 10 min. Centrifuge, and pass a portion of the supernatant through a suitable filter of 0.45-µm pore size.

System suitability

Samples: *System suitability solution* and *Standard solution*. [NOTE—The relative retention times for mebendazole and pimozone are 0.88 and 1.0 respectively.]

Suitability requirements

Resolution: NLT 5.0 between pimozone and mebendazole, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of pimozone from the *Standard solution*

C_S = concentration of USP Pimozone RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of pimozone in the *Sample solution* (mg/mL)

Acceptance criteria

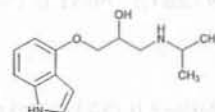
Any individual unspecified degradation product: NMT 0.5%

Total impurities: NMT 0.75%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Mebendazole RS
USP Pimozone RS

Pindolol



$C_{14}H_{20}N_2O_2$ 248.32
2-Propanol, 1-(1*H*-indol-4-yloxy)-3-(1-methylethylamino)-;
1-(Indol-4-yloxy)-3-(isopropylamino)-2-propanol
[13523-86-9].

DEFINITION

Pindolol contains NLT 98.5% and NMT 101.0% of pindolol ($C_{14}H_{20}N_2O_2$), calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile and 0.05 M sodium acetate, previously adjusted with glacial acetic acid to a pH of 5.0 (350:650)

System suitability solution: 0.005 mg/mL each of USP Pindolol RS and indole in *Mobile phase*

Standard stock solution: 1 mg/mL of USP Pindolol RS in *Mobile phase* prepared as follows. To a suitable amount of USP Pindolol RS, add *Mobile phase* to fill about 90% of the total volume, and sonicate for about 5 min to dissolve.

Standard solution: 0.1 mg/mL of USP Pindolol RS from *Standard stock solution* in *Mobile phase*

Sample stock solution: 1 mg/mL of Pindolol in *Mobile phase* prepared as follows. To a suitable amount of Pindolol, add *Mobile phase* to fill about 90% of the total volume, and sonicate for about 5 min to dissolve.

Sample solution: 0.1 mg/mL of Pindolol from *Sample stock solution* in *Mobile phase*

Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 219 nm

Column: 4.6-mm × 15-cm; 3-µm packing L10

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for indole and pindolol are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 7 between indole and pindolol

Column efficiency: NLT 3000 theoretical plates for pindolol

Relative standard deviation: NMT 2% for pindolol

AnalysisSamples: *Standard solution* and *Sample solution*Calculate the percentage of pindolol ($C_{14}H_{20}N_2O_2$) in the portion of Pindolol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response for pindolol from the *Sample solution* r_S = peak response for pindolol from the *Standard solution* C_S = concentration of USP Pindolol RS in the *Standard solution* (mg/mL) C_U = concentration of Pindolol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-Jan-2018)

- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, Chromatographic system, and System suitability: Prepare as directed in the Assay. [NOTE—Decreasing the acetonitrile concentration in *Mobile phase* results in less resolution between pindolol and impurities that elute on the tail of the pindolol peak; increasing the acetonitrile concentration results in less resolution between impurities with longer retention times.]

Sample solution: Use the *Sample stock solution* in the Assay.**Analysis**

Samples: *System suitability solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Pindolol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak area response of each impurity from the *Sample solution* r_S = peak area response for pindolol from the *System suitability solution* C_S = concentration of USP Pindolol RS in the *System suitability solution* (mg/mL) C_U = concentration of Pindolol in the *Sample solution* (mg/mL)**Acceptance criteria**

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **LOSS ON DRYING** (731)

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.

- **USP REFERENCE STANDARDS** (11)

USP Pindolol RS

Pindolol Tablets

» Pindolol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of pindolol ($C_{14}H_{20}N_2O_2$).

Packaging and storage—Preserve in well-closed containers, protected from light.

USP Reference standards (11)—

USP Pindolol RS

Identification—

A: Examine the chromatograms obtained in the test for *Chromatographic purity*: the principal spot obtained from the *Test solution* is similar in R_f value, color, and intensity to that obtained from the *Standard stock solution*.

B: The retention time exhibited by pindolol in the chromatogram of the *Assay preparation* corresponds to that of pindolol in the chromatogram of the *Standard preparation*, as obtained in the Assay.

Dissolution, Procedure for a Pooled Sample (711)—

Medium: 0.1 N hydrochloric acid; 500 mL.

Apparatus 2: 50 rpm.

Time: 15 minutes.

Mobile phase and *Chromatographic system*—Proceed as directed in the Assay.

Standard solution—Dissolve an accurately weighed quantity of USP Pindolol RS in *Dissolution Medium* to obtain a solution having a known concentration of about 0.002/ mg per mL, / being the labeled quantity, in mg, of pindolol in each Tablet. Mix equal volumes of this solution and of *Mobile phase* to obtain the *Standard solution*.

Resolution solution—Dissolve a quantity of nortriptyline hydrochloride in *Standard solution* to obtain a solution having a concentration of about 0.005 mg of nortriptyline hydrochloride per mL.

Test solution—Filter a portion of the solution under test. Mix equal volumes of the filtrate and of *Mobile phase* to obtain the *Test solution*.

Procedure—Proceed as directed for *Procedure* under the Assay. Calculate the quantity of $C_{14}H_{20}N_2O_2$ dissolved by the formula:

$$500C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Pindolol RS in the *Standard solution*; and r_U and r_S are the pindolol peak responses obtained from the *Test solution* and the *Standard solution*, respectively.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{14}H_{20}N_2O_2$ is dissolved in 15 minutes.

Uniformity of dosage units (905): meet the requirements.

Chromatographic purity—[NOTE—Protect solutions and chromatographic plate (after application of solutions) from light.]

p-Dimethylaminobenzaldehyde spray—Dissolve 1 g of *p*-dimethylaminobenzaldehyde in a mixture of 50 mL of hydrochloric acid and 50 mL of alcohol, and mix. [NOTE—Store this solution in a tightly closed, light-resistant container, and discard after 4 weeks.]

Solvent mixture—Prepare a solution of methanol and glacial acetic acid (99:1).

Standard stock solution—Dissolve an accurately weighed quantity of USP Pindolol RS in *Solvent mixture* to obtain a solution containing 5.0 mg per mL.

Standard solution 1—Dilute an accurately measured volume of *Standard stock solution* quantitatively and stepwise with *Solvent mixture* to obtain a solution containing 0.025 mg per mL.

Standard solution 2—Dilute 6.0 mL of *Standard solution 1* with *Solvent mixture* to 10.0 mL, and mix.

Standard solution 3—Dilute 4.0 mL of *Standard solution 1* with *Solvent mixture* to 10.0 mL, and mix.

Standard solution 4—Dilute 2.0 mL of *Standard solution 1* with *Solvent mixture* to 10.0 mL, and mix.

Test solution—[NOTE—Prepare this solution immediately before use, and apply last.] Transfer a portion of powdered Tablets, equivalent to 50 mg of pindolol, to a 50-mL flask, add 10.0 mL of *Solvent mixture*, insert the stopper in the flask, and shake by mechanical means for 15 minutes. Centrifuge a portion of the resultant suspension, and promptly test the clear supernatant.

Procedure—Prepare a lined chromatographic chamber (see *Chromatography* (621)) with a developing solvent consisting of a mixture of methylene chloride, methanol, and formic acid (75:23.5:1.5), and equilibrate for 30 minutes. Separately apply 2-μL portions of the *Standard stock solution*, each of the *Standard solutions*, and the *Test solution* to a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in the chromatographic chamber, and allow the solvent front to move about two-thirds of the length of the plate. Remove the plate from the chamber, immediately spray with the *p*-Dimethylaminobenzaldehyde spray, heat the plate at 60° for 15 minutes, and promptly examine the chromatogram: no individual secondary spot observed in the chromatogram of the *Test solution* is greater in size or intensity than the principal spot observed in the chromatogram of *Standard solution 1*, corresponding to 0.5%, and the total of any such spots observed does not exceed 3.0%. [NOTE—In a valid determination, spots from all solutions must be visible.]

Assay—

Ammonium carbonate solution—Dissolve 300 mg of ammonium carbonate in 50 mL of water, and mix.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, methanol, and Ammonium carbonate solution (475:475:50), making adjustments, if necessary (see *System Suitability under Chromatography* (621)).

Standard preparation—Prepare a solution of USP Pindolol RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

Resolution solution—Dissolve a quantity of nortriptyline hydrochloride in *Standard preparation* to obtain a solution having a concentration of about 0.2 mg of nortriptyline hydrochloride per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of pindolol, to a 100-mL volumetric flask. Add 4 mL of water, and sonicate for 2 minutes, with occasional shaking to disperse the powder. Add 30 mL of *Mobile phase*, sonicate for 15 minutes, and allow to cool. Dilute with *Mobile phase* to volume, mix, and filter. Use the clear filtrate as the *Assay preparation*.

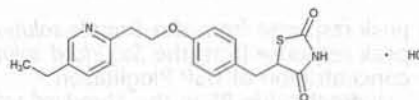
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L16. The flow rate is about 3 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution between pindolol and nortriptyline is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for pindolol and 1.0 for nortriptyline. Calculate the quantity, in mg, of pindolol (C₁₄H₂₀N₂O₂) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Pindolol RS in the *Standard preparation*; and *r_U* and *r_S* are the pindolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pioglitazone Hydrochloride



C₁₉H₂₀N₂O₃S · HCl 392.90
2,4-Thiazolidinedione, 5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-, monohydrochloride, (±)-;
(±)-5-[p-[2-(5-Ethyl-2-pyridyl)ethoxy]benzyl]-2,4-thiazolidinedione monohydrochloride [112529-15-4].

DEFINITION

Pioglitazone Hydrochloride contains NLT 98.0% and NMT 102.0% of C₁₉H₂₀N₂O₃S · HCl, calculated on the anhydrous basis.

IDENTIFICATION

A. INFRARED ABSORPTION (197K)

• **B. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Dissolve 25 mg of Pioglitazone Hydrochloride in 0.5 mL of nitric acid, and add 2 mL of dilute nitric acid. It meets the requirements of the test for *Chloride*.

• **C.** The retention time of the pioglitazone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

PROCEDURE

Mobile phase: Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)

Standard solution: Prepare a 0.5 mg/mL solution of USP Pioglitazone Hydrochloride RS in methanol, and dilute with *Mobile phase* to obtain a solution containing 50 μg/mL of pioglitazone hydrochloride.

System suitability stock solution: 0.5 mg/mL of USP Pioglitazone Hydrochloride RS and 0.13 mg/mL of benzophenone in methanol

System suitability solution: Dilute *System suitability stock solution* with *Mobile phase* to obtain a solution containing 50 μg/mL of pioglitazone hydrochloride and 13 μg/mL of benzophenone.

Sample solution: Prepare a 0.5 mg/mL solution of pioglitazone hydrochloride in methanol, and dilute with *Mobile phase* to obtain a solution containing 50 μg/mL of pioglitazone hydrochloride.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 269 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 25 ± 2.5°

Flow rate: 0.7 mL/min

[NOTE—Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min.]

Injection size: 20 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The approximate relative retention times for pioglitazone and benzophenone are 1.0 and 2.6, respectively.]

Suitability requirements

Tailing factor: NMT 1.5 for pioglitazone and benzophenone, *System suitability solution*

Resolution: NLT 15 between pioglitazone and benzophenone, *System suitability solution*

Relative standard deviation: NMT 2.0% for six replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{19}H_{20}N_2O_3S \cdot HCl$ in the portion of Pioglitazone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (μ g/mL)

C_U = concentration of Pioglitazone Hydrochloride in the *Sample solution* (μ g/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION (281):** NMT 0.1%

Delete the following:

• **HEAVY METALS**

Sodium sulfide solution: 5 g of sodium sulfide in 10 mL of water and 30 mL of glycerin

Magnesium nitrate solution: 100 mg/mL of magnesium nitrate in alcohol

Standard solution: Place 10 mL of *Magnesium nitrate solution* in a platinum or porcelain crucible. Ignite the alcohol to burn. Cool, add 1 mL of sulfuric acid, heat carefully, and ignite at $550 \pm 50^\circ$. Cool and add 3 mL of hydrobromic acid. Proceed as directed from this point under *Sample solution*, adding 1.0 mL of *Standard Lead Solution* (see *Heavy Metals* (231), *Special Reagents*) before adding water to make 50 mL.

Sample solution: Place 1.0 g of pioglitazone hydrochloride in a platinum or porcelain crucible. Mix with 10 mL of *Magnesium nitrate solution*. Ignite the alcohol to burn, and carbonize by gradual heating. Cool, add 1 mL of sulfuric acid, heat carefully, and incinerate by ignition at $550 \pm 50^\circ$. If carbonized substances remain, moisten with a small amount of sulfuric acid, and incinerate by ignition. Cool, dissolve the residue in 3 mL of hydrobromic acid, and evaporate on a water bath to dryness. Wet the residue with 3 drops of hydrochloric acid, add 10 mL of water and dissolve by warming. Add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a pale red color develops. Add 2 mL of 1 N acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL.

Analysis: Add 1 drop of *Sodium sulfide solution* to each of the tubes containing the *Standard solution* and *Sample solution*. Mix thoroughly and allow to stand for 5 min. Compare the colors of both solutions by viewing the tubes downward or transversely against a white

background. The *Sample solution* has no more color than the *Standard solution*.

Acceptance criteria: NMT 10 ppm (Official 1-Jan-2018)

Organic Impurities

• **PROCEDURE**

Mobile phase and System suitability stock solution: Proceed as directed in the Assay.

System suitability solution: Dilute the *System suitability stock solution* with *Mobile phase* to obtain a solution containing 25 μ g/mL of pioglitazone hydrochloride and 6.5 μ g/mL of benzophenone.

Sample solution: 0.2 mg/mL of pioglitazone hydrochloride dissolved in 20% of the final volume with methanol, then diluted with *Mobile phase* to final volume

Standard solution: 1 μ g/mL of pioglitazone hydrochloride prepared by diluting the *Sample solution* with *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 269 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Column temperature: $25 \pm 2.5^\circ$

Flow rate: 0.7 mL/min

[NOTE—Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min.]

Injection size: 40 μ L

Run time: At least four times the retention time of pioglitazone

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for pioglitazone and benzophenone, *System suitability solution*

Resolution: NLT 15 between pioglitazone and benzophenone, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Pioglitazone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of pioglitazone from the *Standard solution*

D = dilution factor used to prepare the *Standard solution*, 0.005

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Hydroxypioglitazone ^a	0.7	0.15
Pioglitazone	1.0	—
Didehydropioglitazone ^b	1.4	0.15

^a (±)-5-[4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl]-5-hydroxythiazolidine-2,4-dione.

^b (Z)-5-[4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzylidene]thiazolidine-2,4-dione.

^c (±)-5-[4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl]-3-[2-(5-ethylpyridin-2-yl)ethyl]thiazolidine-2,4-dione.

Impurity Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
N-Alkylpioglitazone ^c	3.0	0.15
Any other individual impurity	—	0.10

^a (±)-5-[4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl]-5-hydroxythiazolidine-2,4-dione.

^b (Z)-5-[4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzylidene]thiazolidine-2,4-dione.

^c (±)-5-[4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl]-3-[2-(5-ethylpyridin-2-yl)ethyl]thiazolidine-2,4-dione.

SPECIFIC TESTS

- **WATER DETERMINATION**, *Method 1c* (921): NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in well-closed containers, and store at room temperature.
- **USP REFERENCE STANDARDS** (11)
USP Pioglitazone Hydrochloride RS

Pioglitazone Tablets

DEFINITION

Pioglitazone Tablets contain an amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S \cdot HCl$) equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$).

IDENTIFICATION

- **A**. The retention time of the pioglitazone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B. ULTRAVIOLET ABSORPTION**
Sample solution: Dissolve a quantity of finely powdered Tablets in 0.1 N hydrochloric acid to obtain a solution containing 25 µg/mL of pioglitazone. [NOTE—Vigorous shaking and filtration may be needed.]
Acceptance criteria: The UV absorption spectrum exhibits a maximum between 267 and 271 nm.

ASSAY

PROCEDURE

Mobile phase: Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)

Standard solution: Prepare 0.5 mg/mL solution of USP Pioglitazone Hydrochloride RS in methanol, and dilute with *Mobile phase* to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride.

System suitability stock solution: 0.5 mg/mL of USP Pioglitazone Hydrochloride RS and 0.13 mg/mL of benzophenone in methanol

System suitability solution: Dilute the *System suitability stock solution* with *Mobile phase* to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride and 13 µg/mL of benzophenone.

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 23 mg of pioglitazone, to a glass-stoppered flask, and add 50 mL of methanol. Disperse the particles by sonication for about 2 min, then centrifuge. Dilute a portion of the supernatant with *Mobile phase* to obtain a solution having a nominal concentration of 45 µg/mL of pioglitazone.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 269 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 25 ± 2.5°

Flow rate: 0.7 mL/min. [NOTE—Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min.]

Injection size: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The approximate relative retention times for pioglitazone and benzophenone are 1.0 and 2.6, respectively.]

Suitability requirements

Tailing factor: NMT 1.5 for pioglitazone and benzophenone, *System suitability solution*

Resolution: NLT 15 between pioglitazone and benzophenone, *System suitability solution*

Relative standard deviation: NMT 2.0% for six replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of $C_{19}H_{20}N_2O_3S$ in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of pioglitazone, 356.44

M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: Hydrochloric acid and potassium chloride buffer, pH 2.0 [mix 50 mL of 0.2 N hydrochloric acid and 150 mL of potassium chloride solution (150 mg/mL), dilute with water to 1 L, and adjust with 5 N hydrochloric acid to a pH of 2.0]; 900 mL

Apparatus 2: 75 rpm

Time: 15 min

Standard solution: Transfer 23 mg of USP Pioglitazone Hydrochloride RS to a 50-mL volumetric flask, dissolve in 10 mL of methanol, and dilute with *Medium* to volume. Dilute this solution with *Medium* to obtain a final concentration of about L/900, where L is the label claim (mg).

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Detector: UV

Analytical wavelength: 269 nm

Cell: 1 cm

Blank: *Medium*

Calculate the percentage of pioglitazone dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = Tablet label claim (mg)

M_{r1} = molecular weight of pioglitazone, 356.44

M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

V = volume of *Medium* (mL), 900

Tolerances: NLT 80% (Q) of the labeled amount of pioglitazone is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

Procedure for content uniformity

Standard solution: 26 µg/mL of USP Pioglitazone Hydrochloride RS in methanol and 0.1 N hydrochloric acid (9:1)

Sample solution: Transfer 1 Tablet to an appropriate size volumetric flask such that the final concentration does not exceed 0.3 mg of pioglitazone per mL. Add 0.1 N hydrochloric acid at a volume equivalent to 10% of the total volume and shake until the Tablet is completely disintegrated. Add methanol at a volume equivalent to 70% of the total volume and shake vigorously for 10 min. Dilute with methanol to volume, mix well, and centrifuge. Dilute a portion of the supernatant with methanol and 0.1 N hydrochloric acid (9:1) to obtain a solution having a concentration of 24 µg/mL of pioglitazone.

Spectrometric conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV-Vis

Analytical wavelength: 269 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{19}H_{20}N_2O_3S$ in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of pioglitazone, 356.44

M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

IMPURITIES

Organic Impurities

• **PROCEDURE**

Mobile phase and System suitability stock solution: Proceed as directed in the Assay.

Diluent: *Mobile phase* and methanol (4:1)

Standard solution: 1 µg/mL of USP Pioglitazone Hydrochloride RS in *Diluent*. [NOTE—If necessary, dissolve USP Pioglitazone Hydrochloride RS in a minimal amount of methanol and then dilute with *Diluent* to final concentration.]

System suitability solution: Dilute the *System suitability stock solution* with *Mobile phase* to obtain a solution containing 25 µg/mL of pioglitazone hydrochloride and 6.5 µg/mL of benzophenone.

Sample solution: Weigh and finely powder 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 18 mg of pioglitazone, to a 100-mL volumetric flask and add 20 mL of methanol. Disperse the particles by sonication for about 1 min, then dilute with *Mobile phase* to volume, mix well, centrifuge, and use the supernatant.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 269 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 25 ± 2.5°

Flow rate: 0.7 mL/min

[NOTE—Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min.]

Run time: At least 30 min

Injection size: 40 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Tailing factor: NMT 1.5 for pioglitazone and benzophenone, *System suitability solution*

Resolution: NLT 15 between pioglitazone and benzophenone, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—The approximate relative retention times for pioglitazone and benzophenone are 1.0 and 2.6, respectively.]

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of pioglitazone from the *Standard solution*

C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of pioglitazone, 356.44

M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Acceptance criteria

Individual impurities: NMT 0.2%

Total impurities: NMT 0.6%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Pioglitazone Hydrochloride RS

Pioglitazone and Glimepiride Tablets

DEFINITION

Pioglitazone and Glimepiride Tablets contain an amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S \cdot HCl$) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$), and NLT 90.0% and NMT 110.0% of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$).

IDENTIFICATION

• **A. ULTRAVIOLET ABSORPTION**

Sample: Transfer 1 Tablet to a suitable container, and add 0.1 N hydrochloric acid to obtain a final concentration of about 0.1 mg/mL of glimepiride. Shake until the Tablet disintegrates. Pass a 2-mL portion of the resulting suspension through a suitable filter of 0.45-µm pore size. Use the filtrate for the identification of pioglitazone, and use the filter for the identification of glimepiride.

Pioglitazone

Sample solution: Dilute a portion of the filtrate obtained in the *Sample* with 0.1 N hydrochloric acid to obtain a solution containing about 0.03 mg/mL of pioglitazone.

Acceptance criteria: The UV absorption spectrum exhibits a maximum between 267 and 271 nm.

Glimepiride

Sample solution: Wash the filter, as obtained in the *Sample*, with 100 mL of 0.1 N hydrochloric acid, and discard the filtrate. Wash the filter with 20 mL of methanol, and use the filtrate.

Acceptance criteria: The UV absorption spectrum exhibits a maximum between 227 and 231 nm.

- **B.** The retention times of the pioglitazone and glimepiride peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY• **PROCEDURE**

Buffer: 6.9 g/L of monobasic sodium phosphate in water, adjusted with diluted phosphoric acid to a pH of 4.0

Mobile phase: Acetonitrile and *Buffer* (1:1)

Diluent: Acetonitrile and 0.1 N hydrochloric acid (9:1)

Standard stock solution: 0.66 mg/mL of USP Pioglitazone Hydrochloride RS and 0.08 mg/mL of USP Glimepiride RS in *Diluent*

Standard solution: 66 µg/mL of pioglitazone hydrochloride and 8 µg/mL of glimepiride in *Mobile phase* from the *Standard stock solution*

Resolution stock solution: Dilute 1.0 mL of ethyl benzoate with *Mobile phase* to 100 mL. Further dilute 1.0 mL of the resulting solution with *Mobile phase* to 100 mL.

System suitability solution: Transfer 5.0 mL of the *Standard stock solution* and 5.0 mL of the *Resolution stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample stock solution: Transfer 20 Tablets to a suitable container. Add 200.0 mL of *Diluent*, and shake vigorously for at least 20 min. If disintegration is not complete, sonicate until the Tablets are completely disintegrated, and then shake for an additional 10 min. Pass through a suitable filter of 0.2-µm pore size, discarding the first few mL of filtrate. Further dilute 5.0 mL of the filtrate with *Diluent* to 50.0 mL.

Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution with the nominal concentrations listed in *Table 1*.

Table 1

Labeled Amounts of Pioglitazone and Glimepiride (mg/Tablet)	Nominal Concentrations in the <i>Sample solution</i>	
	Pioglitazone (µg/mL)	Glimepiride (µg/mL)
30 and 2	60	4
30 and 4	60	8

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 228 nm

Column: 4.6-mm × 5-cm; 3-µm packing L1

Column temperature: 25 ± 5°

Flow rate: 0.8 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the pioglitazone peak of about 2.3 min.]

Injection volume: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—See *Table 2* for the approximate relative retention times.]

Table 2

Name	Relative Retention Time
Pioglitazone	1.0
Ethyl benzoate	1.7
Glimepiride	2.3

Suitability requirements

Resolution: NLT 4 between pioglitazone and ethyl benzoate; NLT 3 between ethyl benzoate and glimepiride, *System suitability solution*

Relative standard deviation: NMT 1.0% for the pioglitazone peak; NMT 1.0% for the glimepiride peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

r_u = peak response of pioglitazone from the *Sample solution*

r_s = peak response of pioglitazone from the *Standard solution*

C_s = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)

C_u = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of pioglitazone, 356.44

M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Calculate the percentage of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of glimepiride from the *Sample solution*

r_s = peak response of glimepiride from the *Standard solution*

C_s = concentration of USP Glimepiride RS in the *Standard solution* (µg/mL)

C_u = nominal concentration of glimepiride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0% for each of the labeled amounts of pioglitazone and glimepiride

PERFORMANCE TESTS• **DISSOLUTION (711)****Test 1****Pioglitazone**

Medium: Hydrochloric acid buffer pH 2.0 (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 75 rpm

Time: 15 min

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*.

Standard stock solution: 0.37 mg/mL of USP Pioglitazone Hydrochloride RS dissolved first in methanol using 20% of the final volume, and then diluted with *Medium* to volume

Standard solution: Transfer 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with *Medium* to volume.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

System suitability**Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of pioglitazone from the *Sample solution* r_S = peak response of pioglitazone from the *Standard solution* C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (mg/mL) L = labeled amount of pioglitazone (mg/Tablet) V = volume of *Medium*, 900 mL M_{r1} = molecular weight of pioglitazone, 356.44 M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90**Tolerances:** NLT 80% (Q) of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) is dissolved.**Glimepiride****Medium:** pH 6.8 sodium phosphate buffer containing 0.2% of sodium dodecyl sulfate (6.9 g/L of monobasic sodium phosphate, 0.896 g/L of sodium hydroxide, and 2 g/L of sodium dodecyl sulfate in water, adjusted with 1 N sodium hydroxide to a pH of 6.8); 900 mL**Apparatus 2:** 75 rpm**Time:** 15 min**Mobile phase and Chromatographic system:** Proceed as directed in the *Assay*. The flow rate may be adjusted to achieve the retention time of the glimepiride peak of about 5.4 min.**Standard stock solution:** 0.22 mg/mL of USP Glimepiride RS in acetonitrile**Standard solution:** $L/900$ mg/mL of USP Glimepiride RS in *Medium*, where L is the labeled amount of glimepiride, in mg/Tablet, from the *Standard stock solution***Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

 r_U = peak response of glimepiride from the *Sample solution* r_S = peak response of glimepiride from the *Standard solution* C_S = concentration of USP Glimepiride RS in the *Standard solution* (mg/mL) L = labeled amount of glimepiride (mg/Tablet) V = volume of *Medium*, 900 mL**Tolerances:** NLT 80% (Q) of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.**Pioglitazone****Medium:** Hydrochloric acid buffer pH 2.0 (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL, deaerated**Apparatus 2:** 75 rpm**Time:** 30 min**Buffer:** 0.02 M sodium phosphate buffer pH 2.5 (2.75 g/L of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.5)**Solution A:** Acetonitrile and *Buffer* (28:72)**Solution B:** Acetonitrile and *Buffer* (70:30)**Mobile phase:** See *Table 3*.**Table 3**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4.0	0	100
7.0	0	100

Return to original conditions and re-equilibrate the system.

Standard stock solution: 0.2 mg/mL of USP Pioglitazone Hydrochloride RS dissolved first in alcohol using 20% of the final volume, and then diluted with *Medium* to volume**Standard solution:** Transfer 5.0 mL of the *Standard stock solution* to a 25-mL volumetric flask, and dilute with *Medium* to volume.**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 225 nm for pioglitazone (0–4.0 min) and UV 230 nm for glimepiride (4.0–7.0 min)**Column:** 4.6-mm \times 15-cm; 3.5- μ m packing L1**Column temperature:** 30°**Flow rate:** 1.5 mL/min**Injection volume:** 20 μ L**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

 r_U = peak response of pioglitazone from the *Sample solution* r_S = peak response of pioglitazone from the *Standard solution* C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (mg/mL) L = labeled amount of pioglitazone (mg/Tablet) V = volume of *Medium*, 900 mL M_{r1} = molecular weight of pioglitazone, 356.44 M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90**Tolerances:** NLT 80% (Q) of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) is dissolved.**Glimepiride****Medium:** pH 6.8 sodium phosphate buffer containing 0.2% of sodium dodecyl sulfate (6.9 g/L of monobasic sodium phosphate, 0.896 g/L of sodium hydroxide, and 2 g/L of sodium dodecyl sulfate in water, adjusted with 1 N sodium hydroxide to a pH of 6.8); 900 mL**Apparatus 2:** 75 rpm**Time:** 15 min**Mobile phase and Chromatographic system:** Proceed as directed in *Dissolution Test 2*, *Pioglitazone*.

Standard stock solution: 0.2 mg/mL of USP Glimepiride RS in alcohol

Standard solution: $L/900$ mg/mL of USP Glimepiride RS in *Medium*, where L is the labeled amount of glimepiride, in mg/Tablet, from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μ m pore size.

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

r_U = peak response of glimepiride from the *Sample solution*

r_S = peak response of glimepiride from the *Standard solution*

C_S = concentration of USP Glimepiride RS in the *Standard solution* (mg/mL)

L = labeled amount of glimepiride (mg/Tablet)

V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Content Uniformity* for pioglitazone and glimepiride

IMPURITIES

• ORGANIC IMPURITIES: PIOGLITAZONE

Mobile phase: Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)

Diluent: Acetonitrile and 0.1 N hydrochloric acid (9:1)

Standard stock solution: 0.2 mg/mL of USP Pioglitazone Hydrochloride RS in *Diluent*

Standard solution: 2 μ g/mL of USP Pioglitazone Hydrochloride RS in *Mobile phase* from the *Standard stock solution*

Resolution stock solution: Dilute 1.0 mL of ethyl benzoate to 100.0 mL with acetonitrile. Further dilute 1.0 mL of the resulting solution with acetonitrile to 100.0 mL.

System suitability solution: Transfer 2.0 mL of the *Resolution stock solution* into a 100-mL volumetric flask. Add 1.0 mL of the *Standard stock solution*, and dilute with *Mobile phase* to volume.

Sample stock solution: Transfer 10 Tablets to an appropriate volumetric flask such that the nominal glimepiride concentration is 0.4 mg/mL. Add *Diluent* to approximately 80% of the total volume. Shake vigorously for at least 20 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.2- μ m pore size, discarding the first few mL of filtrate.

Sample solution: Transfer a suitable volume of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution containing 240 μ g/mL of pioglitazone.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 269 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Column temperature: $25 \pm 5^\circ$

Flow rate: 0.8 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the pioglitazone peak of about 7 min.]

Injection volume: 40 μ L

Run time: At least 4 times the retention time of the pioglitazone peak

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—Elution order is the pioglitazone peak followed by ethyl benzoate.]

Suitability requirements

Resolution: NLT 10 between pioglitazone and ethyl benzoate, *System suitability solution*

Tailing factor: NMT 1.5 for the pioglitazone peak, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each pioglitazone related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of pioglitazone from the *Standard solution*

C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (μ g/mL)

C_U = nominal concentration of pioglitazone in the *Sample solution* (μ g/mL)

M_{r1} = molecular weight of pioglitazone, 356.44

M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Acceptance criteria

Any individual pioglitazone related impurity: NMT 0.2%

Total pioglitazone related impurities: NMT 0.6% [NOTE—Disregard the peak due to glimepiride, which elutes at about 16.5 min.]

• ORGANIC IMPURITIES: GLIMEPIRIDE

Buffer: 0.007 M sodium phosphate, pH 1.6 (0.97 g/L of monobasic sodium phosphate in water, adjusted with dilute phosphoric acid to a pH of 1.6)

Diluent: Acetonitrile and 0.1 N hydrochloric acid (9:1)

Solution A: Acetonitrile and *Buffer* (52:48)

Solution B: Acetonitrile and *Buffer* (70:30)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
60	0	100
60.1	100	0
70	100	0

Standard stock solution: 0.2 mg/mL of USP Glimepiride RS in *Diluent*

Standard solution: 2 μ g/mL of USP Glimepiride RS in *Solution A* from the *Standard stock solution*

Resolution stock solution: Dilute 1.0 mL of ethyl benzoate with acetonitrile to 100.0 mL. Further dilute 1.0 mL of the resulting solution with acetonitrile to 100.0 mL.

System suitability solution: Transfer 2 mL of the *Resolution stock solution* into a 100-mL volumetric flask, add 1.0 mL of the *Standard stock solution*, and dilute with *Solution A* to volume.

Sample stock solution: Transfer 10 Tablets to an appropriate volumetric flask such that the nominal glimepiride concentration is 0.4 mg/mL. Add *Diluent* to approximately 80% of the total volume. Shake vigorously for at least 20 min, and dilute with *Diluent* to volume.

Pass through a suitable filter of 0.2- μ m pore size, discarding the first few mL of filtrate.

Sample solution: Equivalent to 0.2 mg/mL glimepiride in *Solution A* from the *Sample stock solution*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 228 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Column temperature: 25 \pm 5°

Flow rate: 1.0 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the glimepiride peak of about 25 min.]

Injection volume: 40 μ L

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—Elution order is ethyl benzoate followed by glimepiride.]

Suitability requirements

Resolution: NLT 10 between ethyl benzoate and glimepiride, *System suitability solution*

Tailing factor: NMT 1.5 for glimepiride, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each glimepiride related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of glimepiride from the *Standard solution*

C_S = concentration of USP Glimepiride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of glimepiride in the *Sample solution* (mg/mL)

F = relative response factor for each impurity (see *Table 4*)

Acceptance criteria: See *Table 4*. [NOTE—Disregard the peaks due to inactive ingredients and to pioglitazone that elute before the glimepiride sulfonamide peak.]

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Glimepiride sulfonamide (glimepiride related compound B) ^a	0.2	1.39	1.5
Glimepiride	1.0	1.0	—
Any other glimepiride related individual impurity	—	1.0	0.2
Total glimepiride related impurities	—	—	2.5

^a [p-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl] sulfonamide.

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- LABELING:** When more than one dissolution test is given, the labeling states the *Dissolution Test* used only if *Test 1* is not used.

• USP REFERENCE STANDARDS (11)

USP Glimepiride RS

USP Pioglitazone Hydrochloride RS

Pioglitazone and Metformin Hydrochloride Tablets

DEFINITION

Pioglitazone and Metformin Hydrochloride Tablets contain an amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S \cdot HCl$) equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$), and NLT 95.0% and NMT 105.0% of the labeled amount of metformin hydrochloride ($C_4H_7N_5 \cdot HCl$).

IDENTIFICATION

Change to read:

• A. ULTRAVIOLET ABSORPTION (17U)

[NOTE—The UV spectra of the major peaks of the *Sample solution* and the *Standard solution* as obtained in the *Assay* may also be used to meet the *Acceptance criteria*.] (RB 1-Jun-2016)

Pioglitazone

Sample solution: Transfer a quantity of finely powdered Tablets to a suitable container, and add water to obtain a final concentration of about 0.03 mg/mL of pioglitazone. Sonicate for about 30 s. Pass through a 5-mL portion of the resulting suspension using a suitable filter of 0.45- μ m pore size, then wash the filter with 10 mL of water, and discard the filtrate. Wash the filter with 5 mL of 0.1 N hydrochloric acid, and use the filtrate.

Acceptance criteria: The UV absorption spectrum exhibits a maximum between λ_{265} (RB 1-Jun-2016) and 271 nm.

Metformin hydrochloride

Sample solution: Transfer a quantity of finely powdered Tablets to a suitable container, and add a suitable quantity of water, based on the labeled amount of metformin hydrochloride in the sample, to obtain a final concentration of about 0.4 mg/mL of metformin hydrochloride. Sonicate for about 30 s, and pass through a suitable filter of 0.45- μ m pore size, discarding the first few mL of filtrate. Dilute a portion of the filtrate with water to obtain a solution containing about 8 μ g/mL of metformin hydrochloride.

Acceptance criteria: The UV absorption spectrum exhibits a maximum between 230 and λ_{235} (RB 1-Jun-2016) nm.

- B.** The retention times of the pioglitazone and metformin peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

• PROCEDURE

Mobile phase: 7.2 g/L of sodium dodecyl sulfate in a mixture of 0.05 M monobasic ammonium phosphate and acetonitrile (1:1)

Diluent: Methanol and 0.1 N hydrochloric acid (1:1)

System suitability stock solution: 0.5 mg/mL of *p*-methoxyacetophenone and 0.4 mg/mL of butylparaben in *Diluent*

Pioglitazone standard stock solution: 0.84 mg/mL of USP Pioglitazone Hydrochloride RS in *Diluent*

Mixed standard stock solution: 2.5 mg/mL of USP Metformin Hydrochloride RS and 0.084 mg/mL of USP Pioglitazone Hydrochloride RS in 0.1 N hydrochloric acid from the *Pioglitazone standard stock solution*.

System suitability solution: Transfer 10.0 mL of the *Mixed standard stock solution* and 5.0 mL of the *System suitability stock solution* to a 50-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.

Standard solution: 16.8 µg/mL of USP Pioglitazone Hydrochloride RS and 0.5 mg/mL of USP Metformin Hydrochloride RS in 0.1 N hydrochloric acid from the *Mixed standard stock solution*.

Sample stock solution: Weigh and finely powder NLT 10 Tablets. Transfer an amount of powdered Tablets, equivalent to about 15 mg of pioglitazone, to a 200-mL volumetric flask. Add 120 mL of 0.1 N hydrochloric acid, shake for about 30 min, and then sonicate for about 5 min. Dilute with 0.1 N hydrochloric acid to volume, and mix well. Pass through a suitable filter of 0.45-µm pore size, discarding the first few mL of filtrate.

Sample solution: Transfer a suitable volume of the *Sample stock solution* (see Table 1) to a 50-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.

Table 1

Labeled Amount of Pioglitazone and Metformin Hydrochloride (mg/Tablet)	Volume of Sample Stock Solution Used to Prepare the Sample Solution (mL)	Nominal Concentrations in the Sample Solution	
		Pioglitazone (µg/mL)	Metformin Hydrochloride (mg/mL)
15 and 500	10	15	0.5
15 and 850	5	7.5	0.425

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 255 nm for metformin and *p*-methoxyacetophenone; UV 225 nm for pioglitazone and butylparaben. *If this procedure is used for *Identification A*, use a diode-array detector set at 200–400 nm.

• (RB 1-Jun-2016)

Column: 6.0-mm × 15-cm; 5-µm packing L7

Column temperature: 25 ± 5°

Flow rate: 1 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the metformin peak of about 5 min.]

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for the approximate relative retention times.]

Table 2

Name	Relative Retention Time
Metformin	1.0
<i>p</i> -Methoxyacetophenone	1.2
Pioglitazone	1.8
Butylparaben	2.1

Suitability requirements

Resolution: NLT 2.5 between metformin and *p*-methoxyacetophenone; NLT 2.5 between pioglitazone and butylparaben, *System suitability solution*

Relative standard deviation: NMT 1.0% for the metformin peak; NMT 1.0% for pioglitazone peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pioglitazone (C₁₉H₂₀N₂O₃S) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of pioglitazone from the *Sample solution*

r_S = peak response of pioglitazone from the *Standard solution*

C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of pioglitazone, 356.44

M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Calculate the percentage of the labeled amount of metformin hydrochloride (C₄H₁₁N₅ · HCl) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of metformin from the *Sample solution*

r_S = peak response of metformin from the *Standard solution*

C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of metformin hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0% for each of the labeled amounts of pioglitazone and metformin hydrochloride

PERFORMANCE TESTS

• DISSOLUTION (711)

Test 1

Medium: pH 2.5 McIlvaine buffer (could be prepared by adjusting 0.1 M citric acid with 0.2 M dibasic sodium phosphate to a pH of 2.5); 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Diluent and Mobile phase: Proceed as directed in the *Assay*.

Pioglitazone standard stock solution: 0.37 mg/mL of USP Pioglitazone Hydrochloride RS in *Diluent*

Standard solution: 0.0185 mg/mL of USP Pioglitazone Hydrochloride RS from the *Pioglitazone standard stock solution* and (L/900) mg/mL of USP Metformin Hydrochloride RS in *Medium*, where L is the label claim, in mg/Tablet, of metformin hydrochloride

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system: Proceed as directed in the *Assay*, except use an *Injection volume* of 5 µL.

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.5 for the metformin peak; NMT 2.0 for the pioglitazone peak

Relative standard deviation: NMT 2.0% for the metformin peak; NMT 2.0% for the pioglitazone peak

Analysis

Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response of pioglitazone from the *Sample solution*
 r_S = peak response of pioglitazone from the *Standard solution*
 C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (mg/mL)
 L = label claim of pioglitazone (mg/Tablet)
 V = volume of *Medium*, 900 mL
 M_{r1} = molecular weight of pioglitazone, 356.44
 M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

- r_U = peak response of metformin hydrochloride from the *Sample solution*
 r_S = peak response of metformin hydrochloride from the *Standard solution*
 C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)
 L = label claim of metformin hydrochloride (mg/Tablet)
 V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) is dissolved; NLT 80% (Q) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: pH 2.5 McIlvaine buffer (could be prepared by adjusting 0.1 M citric acid with 0.2 M dibasic sodium phosphate to a pH of 2.5); 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Solution A: 1.4 g/L of dibasic sodium phosphate anhydrous and 1.4 g/L of sodium dodecyl sulfate in water

Solution B: Phosphoric acid and water (50:50)

Mobile phase: Acetonitrile and *Solution A* (34:66). Adjust with *Solution B* to a pH of 7.1.

Diluent A: Acetonitrile and *Medium* (50:50)

Diluent B: Acetonitrile and water (70:30)

Pioglitazone standard stock solution: 0.019 mg/mL of USP Pioglitazone Hydrochloride RS in *Diluent B*. Sonicate as needed to dissolve.

Metformin standard stock solution: 0.92 mg/mL of USP Metformin Hydrochloride RS in *Medium*. Sonicate as needed to dissolve.

Standard solution: 0.003 mg/mL of USP Pioglitazone Hydrochloride RS from the *Pioglitazone standard stock solution* and 0.11 mg/mL of USP Metformin Hydrochloride RS in *Diluent A*

Sample solution: Pass a portion of the solution under test through a suitable filter and dilute with *Diluent A* to a metformin concentration that is similar to the *Standard solution*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm \times 15-cm; 5- μ m packing L1

Temperatures

Autosampler: 5°

Column: 40°

Flow rate: 1 mL/min

Injection volume: 15 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–2.0 for the metformin peak;

0.8–2.0 for the pioglitazone peak

Relative standard deviation: NMT 2.0% for the metformin peak; NMT 2.5% for the pioglitazone peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times (M_{r1}/M_{r2}) \times 100$$

- r_U = peak response of pioglitazone from the *Sample solution*
 r_S = peak response of pioglitazone from the *Standard solution*
 C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (mg/mL)
 L = label claim of pioglitazone (mg/Tablet)
 V = volume of *Medium*, 900 mL
 D = dilution factor of the *Sample solution*
 M_{r1} = molecular weight of pioglitazone, 356.44
 M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times 100$$

- r_U = peak response of metformin hydrochloride from the *Sample solution*
 r_S = peak response of metformin hydrochloride from the *Standard solution*
 C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (mg/mL)
 L = label claim of metformin hydrochloride (mg/Tablet)
 V = volume of *Medium*, 900 mL
 D = dilution factor of the *Sample solution*

Tolerances: NLT 80% (Q) of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) is dissolved; NLT 80% (Q) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905), Content Uniformity:** Meet the requirements for pioglitazone and metformin hydrochloride

IMPURITIES• **ORGANIC IMPURITIES: PIOGLITAZONE**

Mobile phase: Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)

Diluent: Methanol and 0.1 N hydrochloric acid (1:1)

Standard stock solution: 0.2 mg/mL of USP Pioglitazone Hydrochloride RS, dissolved first in methanol using 20% of the final volume, then diluted with *Mobile phase* to volume

System suitability solution: Prepare a solution containing 0.3 mg/mL of benzophenone in methanol. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of the *Standard stock solution*, and dilute with *Mobile phase* to volume. This solution contains 20 μ g/

mL of USP Pioglitazone Hydrochloride RS and 6 µg/mL of benzophenone.

Standard solution: 1 µg/mL of USP Pioglitazone Hydrochloride RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Weigh and finely powder 10 Tablets. Transfer an amount of powdered Tablets, equivalent to about 18 mg of pioglitazone, to a 100-mL volumetric flask, and add 50 mL of *Diluent*. Shake for 30 min, and dilute with *Mobile phase* to volume. Pass through a suitable filter of 0.45-µm pore size, discarding the first few mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 269 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 25 ± 5°

Flow rate: 0.8 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the pioglitazone peak of about 7 min.]

Injection volume: 40 µL

Run time: At least 4 times the retention time of the pioglitazone peak

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—Elution order is the pioglitazone peak followed by benzophenone.]

Suitability requirements

Resolution: NLT 10 between pioglitazone and benzophenone, *System suitability solution*

Tailing factor: NMT 1.5 for the pioglitazone peak, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each pioglitazone related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of pioglitazone from the *Standard solution*

C_S = concentration of USP Pioglitazone Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of pioglitazone, 356.44

M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Acceptance criteria

Any individual pioglitazone related impurity: NMT 0.2%

Total pioglitazone related impurities: NMT 0.6%

[NOTE—Disregard the peaks due to metformin and its impurities that elute before 4.5 min, corresponding to the relative retention time of the pioglitazone peak of about 0.64.]

• ORGANIC IMPURITIES: METFORMIN

Solution A: 1.74 g of sodium 1-pentanesulfonate and 1.15 g of monobasic ammonium phosphate in 1000 mL of water

Solution B: Acetonitrile and water (7:3)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	70	30
15.1	0	100
25	0	100
25.1	100	0
35	100	0

System suitability solution: 5 µg/mL of USP Metformin Hydrochloride RS and 2 µg/mL of melamine in water

Standard solution: 5 µg/mL of USP Metformin Hydrochloride RS in water

Sample solution: Accurately weigh 10 Tablets, and finely powder. Transfer an amount of powdered Tablets, equivalent to about 100 mg of metformin hydrochloride, to a 100-mL volumetric flask, and add 50 mL of water. Shake for 30 min. Dilute with water to volume, and pass through a suitable filter of 0.45-µm pore size, discarding the first few mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5-µm packing L62

Column temperature: 25 ± 5°

Flow rate: 1.0 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the metformin peak of about 8 min.]

Run time: 15 min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for melamine and metformin are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4 between melamine and metformin hydrochloride, *System suitability solution*

Tailing factor: NMT 1.5 for the metformin hydrochloride peak, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each metformin hydrochloride related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of metformin hydrochloride from the *Standard solution*

C_S = concentration of USP Metformin Hydrochloride RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of metformin hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria

Any individual impurity: NMT 0.1%

Total impurities: NMT 0.5%

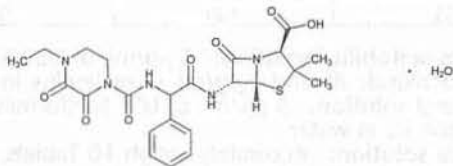
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

- **USP REFERENCE STANDARDS (11)**
USP Metformin Hydrochloride RS
USP Pioglitazone Hydrochloride RS

Piperacillin



$C_{23}H_{27}N_5O_7S \cdot H_2O$ 535.57

$C_{23}H_{27}N_5O_7S$ 517.56

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino]phenylacetyl]amino]-3,3-dimethyl-7-oxo-, monohydrate, [2S-2 α ,5 α ,6 β (S*)];

(2S,5R,6R)-6-[(R)-2-(4-Ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid monohydrate [66258-76-2].

Anhydrous [61477-96-1].

DEFINITION

Piperacillin contains NLT 960 μ g/mg and NMT 1030 μ g/mg of piperacillin ($C_{23}H_{27}N_5O_7S$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, water, 0.2 M monobasic sodium phosphate, and 0.4 M tetrabutylammonium hydroxide (450:447:100:3). Adjust with phosphoric acid to a pH of 5.50.

System suitability solution: 0.1 mg/mL of USP Ampicillin RS and 0.2 mg/mL of USP Piperacillin RS in *Mobile phase*.

Standard solution: 0.4 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Sample solution: 0.4 mg/mL of Piperacillin in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 16 between ampicillin and piperacillin, *System suitability solution*

Tailing factor: NMT 1.2 for the piperacillin peak, *System suitability solution*

Relative standard deviation: NMT 2%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the potency of piperacillin ($C_{23}H_{27}N_5O_7S$) in the portion of Piperacillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (μ g/mg)

Acceptance criteria: 960–1030 μ g/mg on the anhydrous basis

IMPURITIES

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-Jan-2018)

- **AMPICILLIN, PIPERACILLIN PENICILLOIC ACID, PIPERACILLIN RELATED COMPOUND E, AND ACETYLATED PENICILLOIC ACID OF PIPERACILLIN**

Mobile phase, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Standard solution 1: 0.08 mg/mL of USP Ampicillin RS in *Mobile phase*

Standard solution 2: 0.04 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of ampicillin in the portion of Piperacillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of ampicillin from the *Sample solution*

r_S = peak response of ampicillin from *Standard solution 1*

C_S = concentration of USP Ampicillin RS in *Standard solution 1* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of ampicillin in USP Ampicillin RS (μ g/mg)

F = conversion factor, 0.001 mg/ μ g

Calculate the percentages of specified impurities other than ampicillin in the portion of Piperacillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F_1) \times F_2 \times 100$$

r_U = peak response of each specified impurity other than ampicillin from the *Sample solution*

r_S = peak response of piperacillin from *Standard solution 2*

C_S = concentration of USP Piperacillin RS in *Standard solution 2* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (μ g/mg)

F_1 = relative response factor (see *Table 1*)

F_2 = conversion factor, 0.001 mg/ μ g

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin related compound E ^a	0.24	2.4	0.2
Ampicillin	0.31	1.0	0.2
Acetylated penicillic acid of piperacillin ^b	0.37	1.1	0.4
Piperacillin penicillic acid ^c	0.62	0.7	1.0
Piperacillin	1.0	—	—

^a 1-Ethyl-2,3-piperazinedione.

^b (2*R*,4*S*)-3-Acetyl-2-[(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^c (2*R*,4*S*)-2-[(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

• PIPERACILLINYLAMPICILLIN

Mobile phase: Methanol, water, 0.2 M monobasic sodium phosphate, and 0.4 M tetrabutylammonium hydroxide (615:282:100:3). Adjust with phosphoric acid to a pH of 5.50.

Standard solution: 0.04 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Sample solution: 0.4 mg/mL of Piperacillin in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

[NOTE—See Table 2 for the relative retention times.]

Suitability requirements

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of piperacillinylampicillin in the portion of Piperacillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F_1) \times F_2 \times 100$$

r_U = peak response of piperacillinylampicillin from the *Sample solution*

r_S = peak response of piperacillin from the *Standard solution*

C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (μg/mg)

F_1 = relative response factor (see Table 2)

F_2 = conversion factor, 0.001 mg/μg

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin	1.0	—	—
Piperacillinylampicillin ^a	2.55	0.7	2.0
Total impurities ^b	—	—	3.8

^a (2*S*,5*R*,6*R*)-6-[(*R*)-2-[(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^b Total impurities is the sum of all impurities reported in the tests for Ampicillin, Piperacillin Penicillic Acid, Piperacillin Related Compound E, and Acetylated Penicillic Acid of Piperacillin, and Piperacillinylampicillin.

SPECIFIC TESTS

• **WATER DETERMINATION, Method I** <921>: 2.0%–4.0%

• **OPTICAL ROTATION, Specific Rotation** <781S>

Sample solution: 40 mg/mL in methanol

Acceptance criteria: +155° to +175°

• **BACTERIAL ENDOTOXINS TEST** <85>: Where the label states that Piperacillin is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.07 USP Endotoxin Unit/mg of piperacillin.

• **STERILITY TESTS** <71>: Where the label states that Piperacillin is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

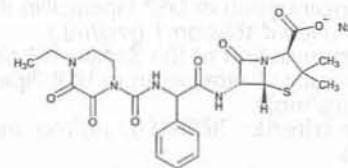
• **USP REFERENCE STANDARDS** <11>

USP Ampicillin RS

USP Endotoxin RS

USP Piperacillin RS

Piperacillin Sodium



$C_{23}H_{26}N_5NaO_7S$ 539.54

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino]phenylacetyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, [2*S*]-[2*α*,5*α*,6*β*(*S*^{*})];

Sodium (2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxo-1-piperazine-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [59703-84-3].

DEFINITION

Piperacillin Sodium has a potency equivalent to NLT 863 μg/mg and NMT 1007 μg/mg of piperacillin ($C_{23}H_{27}N_5O_7S$), calculated on the anhydrous basis.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, and the chromatogram compares qualitatively to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Sodium (191)**

ASSAY• **PROCEDURE**

Mobile phase: Methanol, water, 0.2 M monobasic sodium phosphate, and 0.4 M tetrabutylammonium hydroxide (450:447:100:3). Adjust with phosphoric acid to a pH of 5.50.

System suitability solution: 0.1 mg/mL of USP Ampicillin RS and 0.2 mg/mL of USP Piperacillin RS in *Mobile phase*

Standard solution 1: 0.4 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Sample solution: 0.4 mg/mL of Piperacillin Sodium in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution 1*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 16 between ampicillin and piperacillin, *System suitability solution*

Tailing factor: NMT 1.2 for the piperacillin peak, *System suitability solution*

Relative standard deviation: NMT 2% for the piperacillin peak, *Standard solution 1*

Analysis

Samples: *Standard solution 1* and *Sample solution*

Calculate the potency, in μg/mg, of piperacillin (C₂₃H₂₇N₅O₇S) in the portion of Piperacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

r_U = peak response from the *Sample solution*

r_S = peak response from *Standard solution 1*

C_S = concentration of USP Piperacillin RS in *Standard solution 1* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (μg/mg)

Acceptance criteria: 863–1007 μg/mg on the anhydrous basis

IMPURITIES• **PIPERACILLIN PENICILLOIC ACID AND ACETYLATED PENICILLOIC ACID OF PIPERACILLIN**

Mobile phase, Standard solution 1, and Sample solution: Prepare as directed in the *Assay*.

System suitability solution: 0.1 mg/mL of USP Ampicillin RS and 0.2 mg/mL of USP Piperacillin RS in *Mobile phase*

Standard solution 2: 0.04 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *Standard solution 1* and *System suitability solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 16 between ampicillin and piperacillin, *System suitability solution*

Tailing factor: NMT 1.2 for the piperacillin peak, *System suitability solution*

Relative standard deviation: NMT 2% for the piperacillin peak, *Standard solution 1*

Analysis

Samples: *Sample solution* and *Standard solution 2*

Calculate the percentages of piperacillin penicilloic acid and acetylated penicilloic acid of piperacillin in the portion of Piperacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F_1) \times F_2 \times 100$$

r_U = peak response of piperacillin penicilloic acid or acetylated penicilloic acid of piperacillin from the *Sample solution*

r_S = peak response of piperacillin from *Standard solution 2*

C_S = concentration of USP Piperacillin RS in *Standard solution 2* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (μg/mg)

F_1 = relative response factor (see *Table 1*)

F_2 = conversion factor, 0.001 mg/μg

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin related compound E ^{a,b}	0.24	—	—
Ampicillin	0.31	—	—
Acetylated penicilloic acid of piperacillin ^c	0.37	1.1	1.0
Piperacillin penicilloic acid ^d	0.62	0.7	3.5
Piperacillin	1.0	—	—

^a This impurity is not to be reported.

^b 1-Ethyl-2,3-piperazinedione.

^c (2*R*,4*S*)-3-Acetyl-2-[(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^d (2*R*,4*S*)-2-[(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

SPECIFIC TESTS• **pH (791)**

Sample solution: 400 mg/mL

Acceptance criteria: 5.5–7.5

• **WATER DETERMINATION, Method I (921)**

Test preparation: Proceed as described for hygroscopic substances.

Acceptance criteria: NMT 1.0%

• **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Piperacillin Sodium is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.07 USP Endotoxin Unit/mg of piperacillin.

- **STERILITY TESTS** (71): Where the label states that Piperacillin Sodium is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** (11)
 - USP Ampicillin RS
 - USP Endotoxin RS
 - USP Piperacillin RS

Piperacillin for Injection

DEFINITION

Piperacillin for Injection contains an amount of Piperacillin Sodium equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of piperacillin ($C_{23}H_{27}N_5O_7S$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

Sample: Dissolve 250 mg in water. Add 0.5 mL of 2 N hydrochloric acid and 5 mL of ethyl acetate. Stir, and allow to stand for 10 min in ice water. Pass through a suitable sintered-glass filter, applying suction. Wash the crystals with 5 mL of water and 5 mL of ethyl acetate, then dry in an oven at 60° for 60 min.

Acceptance criteria: Meets the requirements

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, 0.2 M monobasic sodium phosphate, 0.4 M tetrabutylammonium hydroxide, and water (450:100:3:447). Adjust with phosphoric acid to a pH of 5.50.

System suitability solution: 0.1 mg/mL of USP Ampicillin RS and 0.2 mg/mL of USP Piperacillin RS in *Mobile phase*

Standard solution: 0.4 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Sample solution 1 (where it is labeled for use as a single-dose container): Equivalent to 0.4 mg/mL of piperacillin from Piperacillin for Injection constituted as directed below.

Constitute Piperacillin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents using a suitable hypodermic needle and syringe, and dilute with *Mobile phase*.

Sample solution 2 (where the label states the quantity of piperacillin in a given volume of the constituted solution): Equivalent to 0.4 mg/mL of piperacillin from Piperacillin for Injection constituted as directed below. Constitute Piperacillin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Dilute an aliquot of the constituted solution with *Mobile phase*.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 16 between ampicillin and piperacillin, *System suitability solution*

Tailing factor: NMT 1.2 for the piperacillin peak, *System suitability solution*

Relative standard deviation: NMT 2%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of the labeled amount of piperacillin ($C_{23}H_{27}N_5O_7S$) in the container or in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of piperacillin in *Sample solution 1* or *Sample solution 2* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (μg/mg)

F = conversion factor, 0.001 mg/μg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase, System suitability solution, Sample solution 1, Sample solution 2, Chromatographic system, and System suitability: Proceed as directed in the Assay. Evaluate the *Relative standard deviation* using the *Standard solution* prepared in the Assay.

Standard solution: 40 μg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Analysis

Samples: *Sample solution 1* or *Sample solution 2* and *Standard solution*

Calculate the percentage of piperacillin penicilloic acid and acetylated penicilloic acid of piperacillin in the portion of Piperacillin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F_1) \times F_2 \times 100$$

r_U = peak response of piperacillin penicilloic acid or acetylated penicilloic acid of piperacillin from *Sample solution 1* or *Sample solution 2*

r_S = peak response of piperacillin from the *Standard solution*

C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of piperacillin in *Sample solution 1* or *Sample solution 2* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (μg/mg)

F_1 = relative response factor (see *Table 1*)

F_2 = conversion factor, 0.001 mg/μg

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin related compound E ^{a,b}	0.24	—	—
Ampicillin ^a	0.31	—	—
Acetylated penicillic acid of piperacillin ^c	0.37	1.1	1.0
Piperacillin penicillic acid ^d	0.62	0.7	3.5
Piperacillin	1.0	—	—

^a These are process impurities that are listed here for information only; they are controlled in the drug substance and are not to be reported.

^b 1-Ethyl-2,3-piperazinedione.

^c (2*R*,4*S*)-3-Acetyl-2-[(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^d (2*R*,4*S*)-2-[(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

SPECIFIC TESTS

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.07 USP Endotoxin Unit/mg of piperacillin.
- **STERILITY TESTS (71):** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.
- **pH (791):** 4.8–6.8, in a solution of 200 mg/mL of piperacillin
- **WATER DETERMINATION, Method I (921):** NMT 0.9%
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).
- **USP REFERENCE STANDARDS (11)**
USP Ampicillin RS
USP Endotoxin RS
USP Piperacillin RS

Piperacillin and Tazobactam for Injection

DEFINITION

Piperacillin and Tazobactam for Injection contains amounts of Piperacillin Sodium and Tazobactam Sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amounts of piperacillin (C₂₃H₂₇N₅O₇S) and tazobactam (C₁₀H₁₂N₄O₅S), the labeled amounts representing proportions of piperacillin to tazobactam of 8:1. It may contain small amounts of a suitable buffer and stabilizer.

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Buffer: 27.6 g/L of monobasic sodium phosphate

Solution A: 80 mL of 40% aqueous tetrabutylammonium hydroxide diluted with water to 100 mL

Mobile phase: Methanol, water, *Buffer*, and *Solution A* (510:432:50:8). Adjust with phosphoric acid to a pH of 5.5.

Standard solution: 0.1 mg/mL of USP Tazobactam RS and 1 mg/mL of USP Piperacillin RS in *Mobile phase*. Refrigerate the *Standard solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Sample solution: Nominally 0.125 mg/mL of tazobactam and 1 mg/mL of piperacillin from Piperacillin and Tazobactam for Injection in *Mobile phase*. Refrigerate the *Sample solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

Autosampler temperature: 5 ± 3°

System suitability

[NOTE—The relative retention times for tazobactam and piperacillin are 0.36 and 1.0, respectively.]

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for tazobactam and piperacillin

Relative standard deviation: NMT 2.0% for tazobactam and piperacillin

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of piperacillin (C₂₃H₂₇N₅O₇S) in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = peak response of piperacillin from the *Sample solution*

r_S = peak response of piperacillin from the *Standard solution*

C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of piperacillin in the *Sample solution* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (μg/mg)

F = conversion factor, 0.001 mg/μg

Calculate the percentage of the labeled amount of tazobactam (C₁₀H₁₂N₄O₅S) in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of tazobactam from the *Sample solution*

r_S = peak response of tazobactam from the *Standard solution*

C_S = concentration of USP Tazobactam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of tazobactam in the *Sample solution* (mg/mL)

P = potency of tazobactam in USP Tazobactam RS (mg/mg)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

IMPURITIES

• ORGANIC IMPURITIES, PROCEDURE 1

Organic Impurities, Procedure 1 is recommended when the impurity profile includes piperacillin impurities 4, 5, and 6.

Buffer: Dilute the contents of one vial of tetrabutylammonium hydrogen sulfate ion pairing reagent with water to 1 L.

Solution A: Phosphoric acid and water (1:4)

Mobile phase: Acetonitrile and *Buffer* (25:75). Adjust with *Solution A* to a pH of 3.8.

Diluent: Acetonitrile and water (25:75)

Standard stock solution 1: 60 µg/mL of USP Tazobactam Related Compound A RS in *Diluent*

Standard stock solution 2: 0.5 mg/mL of USP Tazobactam RS in *Diluent*

Standard stock solution 3: 1.0 mg/mL of USP Piperacillin RS in acetonitrile and *Diluent* (1:24). Dissolve first in acetonitrile, using about 4% of the final volume, and dilute with *Diluent* to volume.

System suitability solution: 6 µg/mL of tazobactam related compound A from *Standard stock solution 1* and 25 µg/mL of tazobactam from *Standard stock solution 2* in *Diluent*

Standard solution: 25 µg/mL of tazobactam from *Standard stock solution 2* and 0.2 mg/mL of piperacillin from *Standard stock solution 3* in *Mobile phase*. Refrigerate the solution immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Sample solution: Nominally 25 µg/mL of tazobactam and 0.2 mg/mL of piperacillin from Piperacillin and Tazobactam for Injection in *Mobile phase*. Refrigerate the solution immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 3-µm packing L11

Flow rate: 1 mL/min

Injection volume: 20 µL

Autosampler temperature: 5 ± 3°

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 3 between tazobactam related compound A and tazobactam, *System suitability solution*

Tailing factor: NMT 1.8 for tazobactam and piperacillin, *Standard solution*

Relative standard deviation: NMT 2% for tazobactam and piperacillin, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times (F_1/F_2) \times 100$$

r_u = peak response of each impurity from the *Sample solution*

r_s = peak response of piperacillin from the *Standard solution*

C_s = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of piperacillin in the *Sample solution* (mg/mL)

P = potency of USP Piperacillin RS (µg/mg)

F_1 = correction factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor ^a	Acceptance Criteria, NMT (%) ^a
Tazobactam related compound A ^b	0.12	0.75	1.0
Tazobactam	0.25	—	—
Piperacillin impurity 4 ^c	0.31	1.0	1.0
Piperacillin penilloic acid ^{d,e}	0.36	1.0	1.0
Piperacillin penicilloic acid ^{d,f}	0.51	0.56	5.0
Acetylated penicilloic acid of piperacillin ^g	0.55	1.0	1.0
Piperacillin impurity 5 ^c	0.62	1.0	1.0
Piperacillin impurity 6 ^c	0.67	1.0	1.0
Piperacillin	1.0	—	—
Any individual unspecified impurity	—	1.0	1.0
Total impurities ^h	—	—	5.0

^a Calculated relative to the peak area of piperacillin.

^b (2S,3S)-2-Amino-3-methyl-3-sulfinyl-4-(1H-1,2,3-triazol-1-yl)butyric acid.

^c Specified unidentified impurities.

^d This compound has two epimers that usually co-elute but that may be separated as a result of minor changes in the chromatographic conditions.

^e (4S)-2-[[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^f (2R,4S)-2-[(1R)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^g (2R,4S)-3-Acetyl-2-[(1R)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^h Total impurities does not include piperacillin penicilloic acid.

• ORGANIC IMPURITIES, PROCEDURE 2

Organic Impurities, Procedure 2 is recommended when the impurity profile includes piperacillin dimer ethyl ester and piperacillin dimer thiazolamide derivative.

Solution A: 3.12 g/L of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 3.5.

Solution B: Methanol

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	85	15
10	65	35
35	55	45
60	35	65
65	90	10
75	90	10

System suitability solution 1: 10 µg/mL of USP Amoxicillin Related Compound A RS and 6 µg/mL of USP Tazobactam Related Compound A RS in *Solution A*. Refrigerate *System suitability solution 1* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

System suitability solution 2: 0.2 mg/mL each of USP Piperacillin RS and USP Tazobactam RS in a mixture of methanol and *Solution A* (30:70). Prepare the solution by dissolving the compounds in methanol and diluting with *Solution A* to volume. Refrigerate *System suitability solution 2* immediately after preparation and during analysis, using a refrigerated autosampler set at $5 \pm 3^\circ$. Analyze within 24 h of preparation.

Sample solution: Nominally 2 mg/mL of piperacillin and 0.25 mg/mL of tazobactam from Piperacillin and Tazobactam for Injection in *Solution A*. Refrigerate the *Sample solution* immediately after preparation and during analysis, using a refrigerated autosampler set at $5 \pm 3^\circ$. Analyze within 24 h of preparation.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm \times 25-cm; 5- μ m packing L1

Temperatures

Column: 30°

Autosampler: $5 \pm 3^\circ$

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Samples: *System suitability solution 1* and *System suitability solution 2*

Suitability requirements

Resolution: NLT 1.5 between tazobactam related compound A and amoxicillin related compound A, *System suitability solution 1*

Tailing factor: NMT 2.0 for the piperacillin and tazobactam peaks, *System suitability solution 2*

Relative standard deviation: NMT 10.0% for the piperacillin and tazobactam peaks, *System suitability solution 2*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the responses of all peaks from the *Sample solution*

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tazobactam related compound A ^a	0.11	0.3
Amoxicillin related compound A ^b	0.13	0.2
Piperacillin related compound E ^c	0.17	0.8
Tazobactam	0.25	—
Formyl penicillamine ^d	0.34	0.2
Ampicillin	0.45	0.2
Piperazinedione carbonyl D-phenylglycine ^e	0.53	0.2
Acetylated penicilloic acid of piperacillin ^f	0.64	0.5
Piperacillin penicilloic acid, isomer 1 ^g	0.74	0.15
Piperacillin penicilloic acid, isomer 2 ^h	0.78	1.5
L-Piperacillin ^{i,j}	0.81	—
Piperacillin penicilloic acid ^k	0.91	0.5
Piperacillin	1.0	—
Piperacillin methyl ester ^l	1.2	—
Piperacillin dimer ethyl ester ^m	1.3	0.2
Piperacillin dimer thiazolamide derivative ⁿ	1.5	0.2
Piperacillin penicillamide ^o	1.6	0.3
Piperacillin dimer ^p	1.7	0.4
Piperacillinylampicillin ^q	1.9	0.3

^a (2S,3S)-2-Amino-3-methyl-3-sulfinyl-4-[(1H-1,2,3-triazol-1-yl)butyric acid.

^b 6-Aminopenicillanic acid; (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^c 1-Ethylpiperazine-2,3-dione.

^d 2-Formamido-3-mercapto-3-methylbutanoic acid.

^e (R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.

^f N-Acetyl piperacillin open ring; (2R,4S)-3-Acetyl-2-[(1R)-carboxyl-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^g (2S,4S)-2-[(1R)-Carboxyl-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^h Piperacillin open ring; (2R,4S)-2-[(1R)-Carboxyl-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

ⁱ (2S,5R,6R)-6-[(S)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^j Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

^k Piperacillin penicilloic analog; (4S)-2-[(2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^l (2S,5R,6R)-Methyl 6-[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

^m (2S,5R,6R)-Ethyl 6-[(R)-2-[(2S,5R,6R)-6-[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

ⁿ (2R,4S)-2-[(R)-Carboxyl-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-3-[(2R,4S)-2-[(R)-carboxyl-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^o (2S,5R,6R)-6-[(2S,5R,6R)-6-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^p (2R,4S)-2-[(R)-Carboxyl-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-3-[(2S,5R,6R)-6-[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^q Piperacillin amide dimer; (2S,5R,6R)-6-[(R)-2-[(2S,5R,6R)-6-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Table 3 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	0.1
Total impurities	—	4.0

- ^a (2S,3S)-2-Amino-3-methyl-3-sulfino-4-(1H-1,2,3-triazol-1-yl)butyric acid.
^b 6-Aminopenicillanic acid; (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
^c 1-Ethylpiperazine-2,3-dione.
^d 2-Formamido-3-mercapto-3-methylbutanoic acid.
^e (R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.
^f N-Acetyl piperacillin open ring; (2R,4S)-3-Acetyl-2-[(1R)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.
^g (2S,4S)-2-[(1R)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.
^h Piperacillin open ring; (2R,4S)-2-[(1R)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.
ⁱ (2S,5R,6R)-6-[(S)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
^j Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.
^k Piperacillin penilloic analog; (4S)-2-[(2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.
^l (2S,5R,6R)-Methyl 6-[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.
^m (2S,5R,6R)-Ethyl 6-[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.
ⁿ (2R,4S)-2-[(R)-Carboxy[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-3-[(2R,4S)-2-[(R)-carboxy[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxyl)-5,5-dimethylthiazolidine-4-carboxylic acid.
^o (2S,5R,6R)-6-[(2S,5R,6R)-6-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
^p (2R,4S)-2-[(R)-Carboxy[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-3-[(2S,5R,6R)-6-[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxyl)-5,5-dimethylthiazolidine-4-carboxylic acid.
^q Piperacillin amide dimer; (2S,5R,6R)-6-[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

• ORGANIC IMPURITIES, PROCEDURE 3

Organic Impurities, Procedure 3 is recommended when the impurity profile includes piperacillinpenicillenic acid and piperazinedione carbonyl D-phenylglycylglycine.

Buffer: 27.6 g/L of monobasic sodium phosphate dihydrate

Solution A: 0.4 M aqueous tetrabutylammonium hydroxide

Solution B: Methanol, *Solution A*, *Buffer*, and water (275:3:100:622). Adjust with phosphoric acid to a pH of 5.5.

Solution C: Methanol, *Solution A*, *Buffer*, and water (615:3:100:282). Adjust with phosphoric acid to a pH of 5.5.

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution B (%)	Solution C (%)
0	100	0
6	100	0

Table 4 (Continued)

Time (min)	Solution B (%)	Solution C (%)
55	71	29
73	10	90
85	10	90

System suitability solution: 60 µg/mL of USP Piperacillin Related Compound E RS, 0.1 mg/mL of USP Tazobactam Related Compound A RS, and 0.76 mg/mL of USP Tazobactam RS in *Solution C*

Standard solution 1: 6 mg/mL of USP Piperacillin RS in *Solution C*

Standard solution 2: 0.06 mg/mL of USP Piperacillin RS in *Solution C*

Sample solution: Nominally 5.1 mg/mL of piperacillin and 0.64 mg/mL of tazobactam from Piperacillin and Tazobactam for Injection in water. Refrigerate the *Sample solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 4°. Analyze within 10 h of preparation.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Column: 40°

Autosampler: 4°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution 2*

Suitability requirements

Resolution: NLT 1.5 between tazobactam related compound A and piperacillin related compound E, *System suitability solution*

Tailing factor: NMT 2.0 for piperacillin, *Standard solution 2*

Relative standard deviation: NMT 10.0% for the tazobactam peak, *System suitability solution*

Analysis

Samples: *Standard solution 2* and *Sample solution*

Calculate the percentage of each impurity in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of piperacillin from *Standard solution 2*

C_S = concentration of USP Piperacillin RS in *Standard solution 2* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

P = potency of piperacillin in USP Piperacillin RS (µg/mg)

F_1 = correction factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 5*)

Acceptance criteria: See *Table 5*. Disregard peaks that are 0.05 times the response of the peak in *Standard solution 2*. Disregard peaks that elute after piperacillinylampicillin.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin related compound E ^a	0.05	2.4	0.5
Tazobactam related compound A ^b	0.06	0.52	1.0
Tazobactam	0.09	—	—
Formyl penicillamine ^c	0.12	0.31	0.2
Ampicillin	0.14	0.79	0.3
Piperazinedione-carbonyl D-phenylglycine ^d	0.30	1.0	0.5
Piperazinedione-carbonyl D-phenylglycylglycine ^e	0.36	1.0	0.2
Acetylated penicilloic acids of piperacillin ^f	0.57	1.0	0.3
Piperacillinpenicillenic acids ^g	0.60	1.0	0.2
Ampicillin hydantoin analog ^h	0.65	1.0	0.3

^a 1-Ethylpiperazine-2,3-dione.^b (2S,3S)-2-Amino-3-methyl-3-sulfinyl-4-(1*H*-1,2,3-triazol-1-yl)butyric acid.^c 2-Formamido-3-mercapto-3-methylbutanoic acid.^d (R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.^e (R)-2-[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]acetic acid.^f (2*R*,4*S*)-3-Acetyl-2-[(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^g 2-[[[(E)-2-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)(phenyl)methyl]-5-oxooxazol-4(5*H*)-ylidene]methyl]amino]-3-mercapto-3-methylbutanoic acid.^h (2*S*,5*R*,6*R*)-6-(2,5-Dioxo-4-phenylimidazolidin-1-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.ⁱ (2*S*,4*S*)-2-[(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^j (2*R*,4*S*)-2-[(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^k The limit is for the sum of the two epimers of piperacillin open ring.^l (2*S*,5*R*,6*R*)-6-(2-{3-[2-(1-Carboxy-N-ethylformamido)ethyl]ureido}-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.^m (2*S*,5*R*,6*R*)-6-[(*S*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.ⁿ Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.^o (4*S*)-2-[(2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^p The limit is for the sum of the two isomers of piperacillin penilloic analog.^q (2*S*,5*R*,6*R*)-6-[(*R*)-2-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.^r (2*S*,5*R*,6*R*)-6-[(2*R*)-2-[(4*S*)-4-Carboxy-5,5-dimethylthiazolidin-2-yl]-2-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.^s (2*S*,5*R*,6*R*)-6-[(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.^t (2*R*,4*S*)-2-[(*R*)-Carboxy[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-3-[(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^u (2*S*,5*R*,6*R*)-6-[(*R*)-2-[(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Table 5 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin penicilloic acid, isomer 1 ⁱ	0.71	1.0	3.0
Piperacillin penicilloic acid, isomer 2 ^{ik}	0.83	1.0	
Piperacillin oxalamide ^l	0.75	1.0	0.2
L-Piperacillin ^{m,n}	0.80	1.0	—
Piperacillin penilloic acids ^{o,p}	0.87	1.0	1.0
	0.92	1.0	
Piperacillin	1.0	—	—
Piperazinedione-carbonyl D-phenylglycylampicillin ^{n,q}	1.26	1.0	—
Open ring piperacillinylampicillin ^{n,r}	1.36	1.0	—
Piperacillin penicillamide ^s	1.38	1.0	0.2
Piperacillin dimer ^t	1.41	1.0	0.5

^a 1-Ethylpiperazine-2,3-dione.^b (2*S*,3*S*)-2-Amino-3-methyl-3-sulfinyl-4-(1*H*-1,2,3-triazol-1-yl)butyric acid.^c 2-Formamido-3-mercapto-3-methylbutanoic acid.^d (R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.^e (R)-2-[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]acetic acid.^f (2*R*,4*S*)-3-Acetyl-2-[(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^g 2-[[[(E)-2-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)(phenyl)methyl]-5-oxooxazol-4(5*H*)-ylidene]methyl]amino]-3-mercapto-3-methylbutanoic acid.^h (2*S*,5*R*,6*R*)-6-(2,5-Dioxo-4-phenylimidazolidin-1-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.ⁱ (2*S*,4*S*)-2-[(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^j (2*R*,4*S*)-2-[(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^k The limit is for the sum of the two epimers of piperacillin open ring.^l (2*S*,5*R*,6*R*)-6-(2-{3-[2-(1-Carboxy-N-ethylformamido)ethyl]ureido}-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.^m (2*S*,5*R*,6*R*)-6-[(*S*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.ⁿ Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.^o (4*S*)-2-[(2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^p The limit is for the sum of the two isomers of piperacillin penilloic analog.^q (2*S*,5*R*,6*R*)-6-[(*R*)-2-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.^r (2*S*,5*R*,6*R*)-6-[(2*R*)-2-[(4*S*)-4-Carboxy-5,5-dimethylthiazolidin-2-yl]-2-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.^s (2*S*,5*R*,6*R*)-6-[(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.^t (2*R*,4*S*)-2-[(*R*)-Carboxy[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-3-[(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^u (2*S*,5*R*,6*R*)-6-[(*R*)-2-[(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Table 5 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillinylampicillin ^a	1.54	1.0	1.0
Any individual unspecified impurity	—	1.0	0.1

^a 1-Ethylpiperazine-2,3-dione.^b (2S,3S)-2-Amino-3-methyl-3-sulfinyl-4-(1H-1,2,3-triazol-1-yl)butyric acid.^c 2-Formamido-3-mercapto-3-methylbutanoic acid.^d (R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.^e (R)-2-[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]acetic acid.^f (2R,4S)-3-Acetyl-2-[(1R)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^g 2-[[[E]-2-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)(phenyl)methyl]-5-oxooxazol-4(5H)-ylidene]methyl]amino]-3-mercapto-3-methylbutanoic acid.^h (2S,5R,6R)-6-(2,5-Dioxo-4-phenylimidazolidin-1-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.ⁱ (2S,4S)-2-[(1R)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^j (2R,4S)-2-[(1R)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^k The limit is for the sum of the two epimers of piperacillin open ring.^l (2S,5R,6R)-6-(2-[3-(2-(1-Carboxy-N-ethylformamido)ethyl]ureido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.^m (2S,5R,6R)-6-[(S)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.ⁿ Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.^o (4S)-2-[[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.^p The limit is for the sum of the two isomers of piperacillin penilloic analog.^q (2S,5R,6R)-6-[(R)-2-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.^r (2S,5R,6R)-6-[(2R)-2-(2-[(4S)-4-Carboxy-5,5-dimethylthiazolidin-2-yl]-2-[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]acetamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.^s (2S,5R,6R)-6-[(2S,5R,6R)-6-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.^t (2R,4S)-2-[(R)-Carboxy[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-3-[(2S,5R,6R)-6-[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-5,5-dimethylthiazolidine-4-carboxylic acid.^u (2S,5R,6R)-6-[(R)-2-[(2S,5R,6R)-6-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.**• ORGANIC IMPURITIES, PROCEDURE 4**

Organic Impurities, Procedure 4 is recommended when the impurity profile includes piperacillin sulfoxide and piperacillin methyl penicilloate.

Buffer: 4 g/L of monobasic sodium phosphate dihydrate**Solution A:** Acetonitrile and *Buffer* (2:98) adjusted with 1 M sodium hydroxide to a pH of 6.0 ± 0.05**Solution B:** Acetonitrile**Mobile phase:** See Table 6.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	90	10
25	82	18
30	75	25
40	50	50

Table 6 (Continued)

Time (min)	Solution A (%)	Solution B (%)
45	50	50
50	100	0
60	100	0

System suitability solution: 10 µg/mL of USP Amoxicillin Related Compound A RS and 6 µg/mL of USP Tazobactam Related Compound A RS in *Buffer***Standard stock solution:** 1 mg/mL of USP Piperacillin RS and 36 µg/mL of USP Tazobactam RS, prepared as follows. Dissolve suitable amounts of USP Piperacillin RS and USP Tazobactam RS in a small amount of acetonitrile. Dilute with *Buffer* to volume.**Standard solution:** 50 µg/mL of piperacillin and 1.8 µg/mL of tazobactam from *Standard stock solution* in *Buffer***Sample solution:** Nominally 5 mg/mL of piperacillin and 0.625 mg/mL of tazobactam from Piperacillin and Tazobactam for Injection in *Buffer*. Store the *Sample solution* at 2°–8°, and use within 1 h.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 220 nm**Column:** 4.6-mm × 25-cm; 5-µm packing L1**Temperatures****Column:** 30°**Autosampler:** 2°–8°**Flow rate:** 1.5 mL/min**Injection volume:** 20 µL**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 1.5 between tazobactam related compound A and amoxicillin related compound A, *System suitability solution***Tailing factor:** NMT 2.0 for the piperacillin and tazobactam peaks, *Standard solution***Relative standard deviation:** NMT 5.0% for the piperacillin and tazobactam peaks, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity other than tazobactam related compound A in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

 r_U = peak response of each impurity other than tazobactam related compound A from the *Sample solution* r_S = peak response of piperacillin from the *Standard solution* C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL) C_U = nominal concentration of piperacillin in the *Sample solution* (mg/mL) P = potency of piperacillin in USP Piperacillin RS (µg/mg) F = conversion factor, 0.001 mg/µg

Calculate the percentage of tazobactam related compound A in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

 r_U = peak response of tazobactam related compound A from the *Sample solution* r_S = peak response of tazobactam from the *Standard solution*

- C_s = concentration of USP Tazobactam RS in the Standard solution (mg/mL)
 C_u = nominal concentration of tazobactam in the Sample solution (mg/mL)
 P = potency of tazobactam in USP Tazobactam RS (mg/mg)

Acceptance criteria: See Table 7.

Table 7

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tazobactam related compound A ^a	0.08	1.0
Amoxicillin related compound A ^b	0.15	0.2
Piperacillin related compound E ^c	0.18	0.8
Tazobactam	0.25	—
Ampicillin	0.51	0.2
Acetylated penicilloic acid of piperacillin ^d	0.59	0.6
Piperazinedionecarbonyl D-phenylglycine ^e	0.63	0.1
Piperacillin penicilloic acid, isomer 1 ^f	0.65	2.0
Piperacillin penicilloic acid, isomer 2 ^g	0.74	
Ampicillin hydantoin analog, isomer 1 ^h	0.78	0.2
Ampicillin hydantoin analog, isomer 2 ^h	0.80	0.15
Piperacillin sulfoxide ⁱ	0.90	0.15
Piperacillin penilloic analog, isomer 1 ^j	0.94	0.5
Piperacillin penilloic analog, isomer 2 ^j	0.95	
Piperacillin methyl penicilloate ^{k,l}	0.98	—
Piperacillin	1.0	—
Piperacillin dimer ^m	1.2	0.3

^a (2S,3S)-2-Amino-3-methyl-3-sulfinyl-4-(1H-1,2,3-triazol-1-yl)butyric acid.

^b 6-Aminopenicillanic acid; (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^c 1-Ethylpiperazine-2,3-dione.

^d (2R,4S)-3-Acetyl-2-[(1R)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^e (R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.

^f (2S,4S)-2-[(1R)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^g (2R,4S)-2-[(1R)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^h (2S,5R,6R)-6-(2,5-Dioxo-4-phenylimidazolidin-1-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

ⁱ (2S,5R,6R)-6-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4-oxide.

^j (4S)-2-[(2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^k (2R,4S)-2-[(R)-1-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-2-methoxy-2-oxoethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^l Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

^m (2R,4S)-2-[(R)-Carboxy[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-3-[(2S,5R,6R)-6-[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-5,5-dimethylthiazolidine-4-carboxylic acid.

ⁿ (2S,5R,6R)-6-[(R)-2-[(2S,5R,6R)-6-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Table 7 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Piperacillinylampicillin ⁿ	1.31	—
Any individual unspecified impurity	—	0.10

^a (2S,3S)-2-Amino-3-methyl-3-sulfinyl-4-(1H-1,2,3-triazol-1-yl)butyric acid.

^b 6-Aminopenicillanic acid; (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^c 1-Ethylpiperazine-2,3-dione.

^d (2R,4S)-3-Acetyl-2-[(1R)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^e (R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.

^f (2S,4S)-2-[(1R)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^g (2R,4S)-2-[(1R)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^h (2S,5R,6R)-6-(2,5-Dioxo-4-phenylimidazolidin-1-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

ⁱ (2S,5R,6R)-6-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4-oxide.

^j (4S)-2-[(2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^k (2R,4S)-2-[(R)-1-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-2-methoxy-2-oxoethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

^l Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

^m (2R,4S)-2-[(R)-Carboxy[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-3-[(2S,5R,6R)-6-[(R)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-5,5-dimethylthiazolidine-4-carboxylic acid.

ⁿ (2S,5R,6R)-6-[(R)-2-[(2S,5R,6R)-6-[(R)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.08 USP Endotoxin Units in a portion equivalent to 1 mg of a mixture of piperacillin and tazobactam (0.89 and 0.11 mg, respectively).
- **STERILITY TESTS (71):** Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements
- **PH (791)**
Sample solution: Nominally 40 mg/mL of piperacillin
Acceptance criteria: 5.0–7.0
- **WATER DETERMINATION (921), Method I:** NMT 2.5%
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products (1)*.

ADDITIONAL REQUIREMENTS

Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements (659)*, *Injection Packaging, Packaging for constitution* (CN 1-May-2017). Store at controlled room temperature.
- **LABELING:** Label it to indicate its sodium content. If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP REFERENCE STANDARDS (11)**
USP Amoxicillin Related Compound A RS
(2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
C₈H₁₂N₂O₃S 216.26

USP Endotoxin RS
USP Piperacillin RS
USP Piperacillin Related Compound E RS

1-Ethylpiperazine-2,3-dione.

$C_6H_{10}N_2O_2$ 142.16

USP Tazobactam RS

USP Tazobactam Related Compound A RS

(2S,3S)-2-Amino-3-methyl-3-sulfinyl-4-(1H-1,2,3-triazol-1-yl)butyric acid.

$C_7H_{12}N_4O_4S$ 248.26

Piperazine



$C_4H_{10}N_2$ 86.14

Piperazine.

Piperazine [110-85-0].

» Piperazine contains not less than 98.0 percent and not more than 101.0 percent of $C_4H_{10}N_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, protected from light.

USP Reference standards (11)—

USP Piperazine RS

Color of solution—Dissolve 10.0 g in water, and dilute with water to 50.0 mL: the solution has no more color than a standard solution prepared by adding 2.0 mL of ferric chloride CS to water and diluting with water to 50.0 mL, when compared in matched color-comparison tubes.

Identification—

A: Infrared Absorption (197M).

B: In the test for *Chromatographic purity*, the principal spot in the chromatogram of *Test solution 2*, observed after spraying with the ninhydrin solutions, corresponds in R_f value, color, and size to that in the chromatogram of *Standard solution 1*.

Melting range (741): between 109° and 113°.

Water Determination, Method I (921): not more than 2.0%.

Chromatographic purity—

Solvent—Prepare a mixture of 13.5 N ammonium hydroxide and dehydrated alcohol (3:2).

Standard solution 1—Prepare a solution of USP Piperazine RS in *Solvent* containing 10 mg per mL.

Standard solution 2—Prepare a solution of ethylenediamine in *Solvent* containing 0.25 mg per mL.

Standard solution 3—Prepare a solution of triethylenediamine in *Solvent* containing 0.25 mg per mL.

Resolution solution—Prepare a solution in *Solvent* containing 0.25 mg of triethylenediamine and 10 mg of USP Piperazine RS per mL.

Test solution 1—Prepare a solution of Piperazine in *Solvent* containing 100 mg per mL.

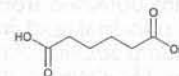
Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of *Solvent*.

Procedure—Apply separate 5-μL portions of *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, *Resolution solution*, *Test solution 1*, and *Test solution 2* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatograms in a

solvent system consisting of a freshly prepared mixture of acetone and 13.5 N ammonium hydroxide (80:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 105°. Spray the plate with a 0.3% (w/v) solution of ninhydrin in a mixture of butyl alcohol and glacial acetic acid (100:3). Spray the plate again with a 0.15% (w/v) solution of ninhydrin in dehydrated alcohol, dry the plate at 105° for 10 minutes, and examine the plate: any secondary spot in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 2* (0.25%). Spray the plate with 0.1 N iodine TS, allow to stand for 10 minutes, and examine the plate: any spot corresponding to triethylenediamine in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 3* (0.25%). In a valid test, the chromatogram obtained from the *Resolution solution* shows a spot due to triethylenediamine clearly separated from the principal spot. Disregard any spot at the origin of any chromatogram.

Assay—Weigh accurately about 150 mg of Piperazine, and dissolve in 75 mL of glacial acetic acid. Titrate potentiometrically with 0.1 N perchloric acid VS, using a silver-glass electrode system. As the endpoint is approached, warm the solution to 60° to 70°, then complete the titration. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 4.307 mg of $C_4H_{10}N_2$.

Piperazine Adipate



$C_4H_{10}N_2 \cdot C_6H_{10}O_4$ 232.28

Piperazine, compound with 1,4-butanediacycarboxylic acid (1:1).

Piperazine, compound with hexanedioic acid (1:1) [142-88-1].

» Piperazine Adipate contains not less than 98.0 percent and not more than 101.0 percent of $C_4H_{10}N_2 \cdot C_6H_{10}O_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers, and store at room temperature.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Piperazine Adipate RS

Identification—

A: Infrared Absorption (197K).

B: In the test for *Chromatographic purity*, the principal spot in the chromatogram obtained from *Test solution 2* observed after spraying with the ninhydrin solutions corresponds in R_f value, color, and size to that in the chromatogram obtained from *Standard solution 1*.

C: To 10 mL of a 1 in 20 solution of it add 5 mL of hydrochloric acid, and extract with three 10-mL portions of ether. Evaporate the combined ether extracts to dryness, wash the residue with water, and dry at 105°: the residue of adipic acid so obtained melts at between 150° and 154°.

Water Determination, Method I (921): not more than 0.5%.

Residue on ignition (281): not more than 0.1%.

Chromatographic purity—

Solvent—Prepare a mixture of 13.5 N ammonium hydroxide and dehydrated alcohol (3:2).

Test solution 1—Prepare a solution of Piperazine Adipate in *Solvent* containing 100 mg per mL.

Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of *Solvent*.

Standard solution 1—Prepare a solution of USP Piperazine Adipate RS in *Solvent* containing 10 mg per mL.

Standard solution 2—Prepare a solution of ethylenediamine in *Solvent* containing 0.25 mg per mL.

Standard solution 3—Prepare a solution of triethylenediamine in *Solvent* containing 0.25 mg per mL.

Resolution solution—Prepare a solution in *Solvent* containing 0.25 mg of triethylenediamine and 10 mg of Piperazine Adipate per mL.

Procedure—Separately apply 5-μL portions of *Test solution 1*, *Test solution 2*, *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and the *Resolution solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a freshly prepared mixture of acetone and 13.5 N ammonium hydroxide (80:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 105°. Spray the plate with a 0.3% solution of ninhydrin in a mixture of butyl alcohol and glacial acetic acid (100:3). Spray the plate again with a 0.15% solution of ninhydrin in dehydrated alcohol, dry the plate at 105° for 10 minutes, and examine the plate: any secondary spot in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 2* (0.25%). Spray the plate with 0.1 N iodine TS, allow to stand for 10 minutes, and examine the plate: any spot corresponding to triethylenediamine in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 3* (0.25%). In a valid test, the chromatogram obtained from the *Resolution solution* shows a spot due to triethylenediamine clearly separated from the principal spot. Disregard any spot at the origin of any chromatogram.

Assay—Dissolve about 100 mg of Piperazine Adipate, accurately weighed, in 10 mL of glacial acetic acid, heating gently if necessary. Add 60 mL of glacial acetic acid and 0.25 mL of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid until the brownish-yellow color of the solution turns green. Each mL of 0.1 N perchloric acid is equivalent to 11.61 mg of $C_4H_{10}N_2 \cdot C_6H_8O_7$.

$(C_4H_{10}N_2)_3 \cdot 2C_6H_8O_7$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Piperazine Citrate RS

Identification—

A: Infrared Absorption (197K).

B: In the test for *Chromatographic purity*, the principal spot in the chromatogram of *Test solution 2*, observed after spraying with the ninhydrin solutions, corresponds in R_f value, color, and size to that in the chromatogram of *Standard solution 1*.

C: It responds to the tests for *Citrate* (191).

Water Determination, Method I (921): not more than 12.0%.

Chromatographic purity—

Solvent—Prepare a mixture of 13.5 N ammonium hydroxide and dehydrated alcohol (3:2).

Standard solution 1—Prepare a solution of USP Piperazine Citrate RS in *Solvent* containing 10 mg per mL.

Standard solution 2—Prepare a solution of ethylenediamine in *Solvent* containing 0.25 mg per mL.

Standard solution 3—Prepare a solution of triethylenediamine in *Solvent* containing 0.25 mg per mL.

Test solution 1—Prepare a solution of Piperazine Citrate in *Solvent* containing 100 mg per mL.

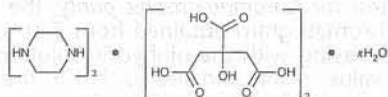
Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of *Solvent*.

Resolution solution—Prepare a solution in *Solvent* containing 0.25 mg of triethylenediamine and 10 mg of Piperazine Citrate per mL.

Procedure—Apply separate 5-μL portions of *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, *Resolution solution*, *Test solution 1*, and *Test solution 2* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a freshly prepared mixture of acetone and 13.5 N ammonium hydroxide (80:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 105°. Spray the plate with a 0.3% (w/v) solution of ninhydrin in a mixture of butyl alcohol and glacial acetic acid (100:3). Spray the plate again with a 0.15% (w/v) solution of ninhydrin in dehydrated alcohol, dry the plate at 105° for 10 minutes, and examine the plate: any secondary spot in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 2* (0.25%). Spray the plate with 0.1 N iodine TS, allow to stand for 10 minutes, and examine the plate: any spot corresponding to triethylenediamine in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 3* (0.25%). In a valid test, the chromatogram obtained from the *Resolution solution* shows a spot due to triethylenediamine clearly separated from the principal spot. Disregard any spot at the origin of any chromatogram.

Assay—Dissolve about 200 mg of Piperazine Citrate, accurately weighed, in 100 mL of glacial acetic acid, warming slightly if necessary to effect solution. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 10.71 mg of $(C_4H_{10}N_2)_3 \cdot 2C_6H_8O_7$.

Piperazine Citrate



$(C_4H_{10}N_2)_3 \cdot 2C_6H_8O_7 \cdot xH_2O$ (anhydrous) 642.65
Piperazine, 2-hydroxy-1,2,3-propanetricarboxylate (3:2),
hydrate.

Piperazine citrate (3:2) hydrate [41372-10-5].
Anhydrous 642.66 [144-29-6].

» Piperazine Citrate contains not less than 98.0 percent and not more than 100.5 percent of

Piperazine Citrate Syrup

» Piperazine Citrate Syrup is prepared from Piperazine Citrate or from Piperazine to which an equivalent amount of Citric Acid is added. It contains an amount of piperazine citrate equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of piperazine hexahydrate ($C_4H_{10}N_2 \cdot 6H_2O$).

Packaging and storage—Preserve in tight containers.

Identification—

A: To 2 mL of Syrup add 5 mL of 3 N hydrochloric acid, then add, with stirring, 1 mL of sodium nitrite solution (1 in 2). Chill in an ice bath for 15 minutes, stirring if necessary, to induce crystallization, filter the precipitate on a sintered-glass funnel, wash with 10 mL of cold water, and dry at 105°: the *N,N'*-dinitrosopiperazine so obtained melts between 156° and 160°.

B: It responds to the tests for *Citrate* (191).

Assay—Determine the specific gravity of Syrup, and transfer an accurately weighed portion of the Syrup, equivalent to about 200 mg of piperazine citrate, to a 250-mL beaker. Add 10 mL of water and 75 mL of trinitrophenol TS, stir well, and allow to stand in a refrigerator for not less than 2 hours. Collect the precipitate in a tared filtering crucible, wash with five 10-mL portions of dehydrated alcohol, and dry at 105° to constant weight. [Caution—Picrates may explode.] The weight of the dipicrate, multiplied by 0.3568, gives the equivalent of $C_4H_{10}N_2 \cdot 6H_2O$ in the portion of Syrup taken.

Piperazine Citrate Tablets

» Piperazine Citrate Tablets contain an amount of piperazine citrate equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of piperazine hexahydrate ($C_4H_{10}N_2 \cdot 6H_2O$).

Packaging and storage—Preserve in tight containers.

Identification—

A: Finely powder a number of Tablets, equivalent to about 200 mg of piperazine citrate, mix with 5 mL of 3 N hydrochloric acid, and filter. To the filtrate add, with stirring, 1 mL of sodium nitrite solution (1 in 2). Chill in an ice bath for 15 minutes, stirring if necessary, to induce crystallization, filter the precipitate on a sintered-glass funnel, wash with 10 mL of cold water, and dry at 105°: the *N,N'*-dinitrosopiperazine so obtained melts between 156° and 160°.

B: To a quantity of powdered Tablets, equivalent to about 500 mg of piperazine citrate, add 10 mL of water, shake, and filter: the filtrate responds to the tests for *Citrate* (191).

Dissolution, Procedure for a Pooled Sample (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

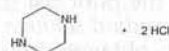
Procedure—Determine the amount of piperazine hexahydrate ($C_4H_{10}N_2 \cdot 6H_2O$) dissolved, employing the procedure set forth in the Assay, making any necessary modifications.

Tolerances—Not less than 75% (Q) of the labeled amount of $C_4H_{10}N_2 \cdot 6H_2O$ is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Assay—Weigh and finely powder not less than 20 Tablets. Shake an accurately weighed portion of the powder, equivalent to about 200 mg of piperazine citrate, for 1 hour with 10 mL of a mixture of 1 part of 3 N hydrochloric acid and 3 parts of water, filter, and wash the residue with two 10-mL portions of water. To the combined extract and washings add 75 mL of trinitrophenol TS, and proceed as directed in the Assay under *Piperazine Citrate Syrup*, beginning with "stir well."

Piperazine Dihydrochloride



$C_4H_{10}N_2 \cdot 2HCl \cdot xH_2O$ 159.06 (anhydrous)
Piperazine dihydrochloride hydrate [142-64-3].

» Piperazine Dihydrochloride contains not less than 98.5 percent and not more than 100.5 percent of $C_4H_{10}N_2 \cdot 2HCl$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers, and store at room temperature.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—

USP Piperazine Dihydrochloride RS.

Identification—

A: Infrared Absorption (197K)—

Test specimen: previously dried at 105° for 3 hours.

B: In the test for *Chromatographic purity*, the principal spot in the chromatogram obtained from *Test solution 2*, observed after spraying with the ninhydrin solutions, corresponds in *R_f* value, color, and size to that in the chromatogram obtained from *Standard solution 1*.

C: It meets the requirements of the test for *Chloride* (191).

pH (791): between 3.0 and 3.4, in a solution (1 in 20).

Water Determination, Method I (921): not more than 10.0%.

Residue on ignition (281): not more than 0.1%.

Chromatographic purity—

Solvent—Prepare a mixture of 13.5 N ammonium hydroxide and dehydrated alcohol (3:2).

Test solution 1—Prepare a solution of Piperazine Dihydrochloride in *Solvent* containing 100 mg per mL.

Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of *Solvent*.

Standard solution 1—Prepare a solution of USP Piperazine Dihydrochloride RS in *Solvent* containing 10 mg per mL.

Standard solution 2—Prepare a solution of ethylenediamine in *Solvent* containing 0.25 mg per mL.

Standard solution 3—Prepare a solution of triethylenediamine in *Solvent* containing 0.25 mg per mL.

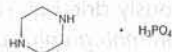
Resolution solution—Prepare a solution in *Solvent* containing 0.25 mg of triethylenediamine and 10 mg of Piperazine Dihydrochloride per mL.

Procedure—Separately apply 5-μL portions of *Test solution 1*, *Test solution 2*, *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and the *Resolution solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic

silica gel. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a freshly prepared mixture of acetone and 13.5 N ammonium hydroxide (80:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plates from the developing chamber, mark the solvent front, and dry the plate at 105°. Spray the plate with a 0.3% solution of ninhydrin in a mixture of butyl alcohol and glacial acetic acid (100:3). Spray the plate again with a 0.15% solution of ninhydrin in dehydrated alcohol, dry the plate at 105° for 10 minutes, and examine the plate: any secondary spot in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 2* (0.25%). Spray the plate with 0.1 N iodine TS, allow to stand for 10 minutes, and examine the plate: any spot corresponding to triethylenediamine in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 3* (0.25%). In a valid test, the chromatogram obtained from the *Resolution solution* shows a spot due to triethylenediamine clearly separated from the principal spot. Disregard any spot at the origin of any chromatogram.

Assay—Dissolve about 140 mg of Piperazine Dihydrochloride in 4 mL of ethylene glycol using a 150-mL beaker. Add 25 mL of glacial acetic acid containing 1.2 g of mercuric acetate, rinsing the walls of the beaker with a small amount of the glacial acetic acid. Add 0.25 mL of *p*-naphtholbenzene TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 7.953 mg of $C_4H_{10}N_2 \cdot 2HCl$.

Piperazine Phosphate



$C_4H_{10}N_2 \cdot H_3PO_4 \cdot H_2O$ 202.15
 Piperazine phosphate (1:1), monohydrate.
 Piperazine phosphate (1:1), monohydrate [18534-18-4].
 Anhydrous 184.13 [14538-56-8].

» Piperazine Phosphate contains not less than 98.5 percent and not more than 100.5 percent of $C_4H_{10}N_2 \cdot H_3PO_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, and store at room temperature.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—
 USP Piperazine Phosphate RS

Identification—

A: Infrared Absorption (197K)—

Test specimen: previously dried at 105° for 3 hours.

B: In the test for *Chromatographic purity*, the principal spot in the chromatogram obtained from *Test solution 2*, observed after spraying with the ninhydrin solutions, corresponds in R_f value, color, and size to that in the chromatogram obtained from *Standard solution 1*.

C: It meets the requirements of the test for *Phosphate* (191).

pH (791): between 6.0 and 6.5, in a solution (1 in 100).

Water, Method I (921): between 8.0% and 9.5%.

Chromatographic purity—

Solvent—Prepare a mixture of 13.5 N ammonium hydroxide and dehydrated alcohol (3:2).

Test solution 1—Prepare a solution of Piperazine Phosphate in *Solvent* containing 100 mg per mL.

Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of *Solvent*.

Standard solution 1—Prepare a solution of USP Piperazine Phosphate RS in *Solvent* containing 10 mg per mL.

Standard solution 2—Prepare a solution of ethylenediamine in *Solvent* containing 0.25 mg per mL.

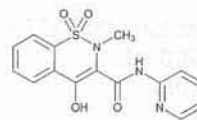
Standard solution 3—Prepare a solution of triethylenediamine in *Solvent* containing 0.25 mg per mL.

Resolution solution—Prepare a solution in *Solvent* containing 0.25 mg of triethylenediamine and 10 mg of Piperazine Phosphate per mL.

Procedure—Separately apply 5-μL portions of *Test solution 1*, *Test solution 2*, *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and the *Resolution solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a freshly prepared mixture of acetone and 13.5 N ammonium hydroxide (80:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 105°. Spray the plate with a 0.3% solution of ninhydrin in a mixture of butyl alcohol and glacial acetic acid (100:3). Spray the plate again with a 0.15% solution of ninhydrin in dehydrated alcohol, dry the plate at 105° for 10 minutes, and examine the plate: any secondary spot in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 2* (0.25%). Spray the plate with 0.1 N iodine TS, allow to stand for 10 minutes, and examine the plate: any spot corresponding to triethylenediamine in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 3* (0.25%). In a valid test, the chromatogram obtained from the *Resolution solution* shows a spot due to triethylenediamine clearly separated from the principal spot. Disregard any spot at the origin of any chromatogram.

Assay—Dissolve about 200 mg of Piperazine Phosphate in 4 mL of ethylene glycol using a 150-mL beaker. Add 25 mL of glacial acetic acid, rinsing the walls of the beaker with a small amount of the glacial acetic acid. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 7.953 mg of $C_4H_{10}N_2 \cdot 2HCl$.

Piroxicam



$C_{15}H_{13}N_3O_4S$ 331.35
 2*H*-1,2-Benzothiazine-3-carboxamide, 4-hydroxy-2-methyl-*N*-2-pyridinyl-, 1,1-dioxide;
 4-Hydroxy-2-methyl-*N*-2-pyridyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide [36322-90-4].

DEFINITION

Piroxicam contains NLT 97.0% and NMT 103.0% of piroxicam ($C_{15}H_{13}N_3O_4S$).

IDENTIFICATION

- **A. INFRARED ABSORPTION (197M):** Do not dry specimens.
- **B. ULTRAVIOLET ABSORPTION (197U)**

Solution: 10 µg/mL

Medium: Hydrochloric acid in methanol (1 in 1200)

Acceptance criteria: Meets the requirements

- **C. THIN-LAYER CHROMATOGRAPHY**

Diluent: Chloroform and methanol (1:1)

Standard solution: 1 mg/mL of USP Piroxicam RS in Diluent

Sample solution: 1 mg/mL in Diluent

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Absorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 20 µL

Developing solvent system: Toluene and glacial acetic acid (95:5)

Analysis

Samples: Standard solution and Sample solution

Allow the spots to dry, and develop the chromatogram in the Developing solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and air-dry. Place the plate in the developing chamber, and develop as before. Remove the plate from the chamber, mark the solvent front, and air-dry. Locate the spots on the plate by viewing under short-wavelength UV light.

Acceptance criteria: The R_f value of the principal spot of the Sample solution corresponds to that of the Standard solution.

ASSAY• **PROCEDURE**

Solution A: Dissolve 7.72 g of anhydrous citric acid in 400 mL of water.

Solution B: Dissolve 5.35 g of dibasic sodium phosphate in 100 mL of water.

Solution C: Add Solution B to Solution A, and dilute with water to 1000 mL.

Mobile phase: Methanol and Solution C (450:550)

Diluent: 0.01 N methanolic hydrochloric acid

Standard stock solution: 0.25 mg/mL of USP Piroxicam RS in Diluent

Standard solution: 0.05 mg/mL of USP Piroxicam RS prepared as follows. Transfer 10.0 mL of Standard stock solution to a 50-mL volumetric flask, add about 25 mL of Diluent and 10.0 mL of water, and dilute with Diluent to volume.

Sample stock solution: 0.5 mg/mL of Piroxicam in Diluent

Sample solution: Transfer 10.0 mL of Sample stock solution to a second 100-mL volumetric flask, add about 50 mL of Diluent and 20.0 mL of water, and dilute with Diluent to volume.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 25 µL

System suitability

Sample: Standard solution

Suitability requirements

Column efficiency: NLT 500 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of piroxicam ($C_{15}H_{13}N_3O_4S$) in the portion of Piroxicam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of USP Piroxicam RS in the Standard solution (mg/mL)

C_U = concentration of Piroxicam in the Sample solution (mg/mL)

Acceptance criteria: 97.0%–103.0%

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.3%

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 50 ppm (Official 1-Jan-2018)

SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

- **USP REFERENCE STANDARDS (11)**
USP Piroxicam RS

Piroxicam Capsules**DEFINITION**

Piroxicam Capsules contain NLT 92.5% and NMT 107.5% of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$).

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Chloroform and methanol (1:1)

Standard solution: 1 mg/mL of USP Piroxicam RS in Diluent

Sample solution: Dissolve a portion of the contents of Capsules in Diluent to obtain a solution containing about 1 mg/mL. Shake by mechanical means for 10 min, and filter a portion. Use the filtrate for the analysis.

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Absorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 20 µL

Developing solvent system: Toluene and glacial acetic acid (95:5)

Analysis

Samples: Standard solution and Sample solution

Allow the spots to dry, and develop the chromatogram in the Developing solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the devel-

oping chamber, and air-dry. Place the plate in the developing chamber, and develop as before. Remove the plate from the chamber, mark the solvent front, and air-dry. Locate the spots on the plate by viewing under short-wavelength UV light.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

PROCEDURE

Solution A: Dissolve 7.72 g of anhydrous citric acid in 400 mL of water.

Solution B: Dissolve 5.35 g of dibasic sodium phosphate in 100 mL of water.

Solution C: Add *Solution B* to *Solution A*, and dilute with water to make 1000 mL.

Mobile phase: Methanol and *Solution C* (450:550)

Diluent: 0.01 N methanolic hydrochloric acid

Standard stock solution: 0.25 mg/mL of USP Piroxicam RS in *Diluent*

Standard solution: 0.05 mg/mL of USP Piroxicam RS prepared as follows. Transfer 10.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add 25 mL of *Diluent* and 10.0 mL of water, and dilute with *Diluent* to volume.

Sample stock solution: Transfer, as completely as possible, the contents of NLT 20 Capsules to a suitable tared container, and determine the average weight per capsule. Mix the combined contents, and transfer an amount nominally equivalent to about 50 mg of piroxicam to a 100-mL volumetric flask. Add about 70 mL of *Diluent*, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume, and mix. Centrifuge a portion of this mixture to obtain a clear solution.

Sample solution: Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add about 50 mL of *Diluent* and 20.0 mL of water, dilute with *Diluent* to volume, and mix.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 500 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Piroxicam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of piroxicam in the *Sample solution* (mg/mL)

Acceptance criteria: 92.5%–107.5%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: Simulated gastric fluid TS, prepared without pepsin; 900 mL

Apparatus 1: 50 rpm

Time: 45 min

Standard stock solution: 0.5 mg/mL of USP Piroxicam RS in methanol

Standard solution: A known concentration of USP Piroxicam RS in *Medium* from *Standard stock solution*. Dilute with *Medium*, if necessary.

Sample solution: Filter a portion of the solution under test, suitably dilute with *Medium*, if necessary.

[NOTE—Use a suitable filter that does not adsorb piroxicam.]

Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 333 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$) dissolved by comparing the UV absorbance of *Sample solution* with that of *Standard solution*.

Tolerances: NLT 75% (Q) of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 8.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**
USP Piroxicam RS

Piroxicam Compounded Cream

DEFINITION

Piroxicam Compounded Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$).

Prepare Piroxicam Compounded Cream as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Piroxicam	3 g
White Petrolatum	25 g
Stearyl Alcohol	15 g
Propylparaben	0.06 g
Methylparaben	0.15 g
Propylene Glycol	12.0 g
Sodium Lauryl Sulfate	1 g
Sodium Hydroxide, 1 N	2.5 mL
Purified Water, a sufficient quantity to make	100 g

In an appropriate container (final weight tared), mix the *White Petrolatum* and *Stearyl Alcohol* together, and heat to $80 \pm 5^\circ$ to form a clear oil phase. In a separate container, mix the *Propylparaben*, *Methylparaben*, *Propylene Glycol*, *Sodium Lauryl Sulfate*, and about 30 mL of *Purified Water* together, and heat to $80 \pm 5^\circ$ to form a clear aqueous phase. Add the aqueous phase to the oil phase with continuous stirring, and allow it to cool to 50° to form an emulsion. In a mortar, triturate the *Piroxicam* with the *Sodium Hydroxide* to form a suspension. Using additional *Purified Water* to rinse the mortar, add the piroxicam suspension to the previously prepared emulsion, transferring the suspension stepwise and quantitatively to the emulsion. Add sufficient *Purified Water* with stirring to bring to final weight. Package, and label.

ASSAY**• PROCEDURE**

Solution A: 2.7 g/L of citric acid and 5.4 g/L of dibasic sodium phosphate in *Purified Water*. Pass through a filter of 0.45- μ m pore size.

Mobile phase: Methanol and *Solution A* (50:50). Filter, and degas.

Diluent: 0.01 N methanolic hydrochloric acid prepared by diluting 0.9 mL of hydrochloric acid with methanol to a final volume of 1 L

Standard solution: Dissolve an accurately weighed quantity of USP Piroxicam RS in 2 mL of chloroform, and dilute with *Diluent* to obtain a solution with a nominal concentration of about 50 μ g/mL of piroxicam.

Sample solution: Add 340 mg of Cream to 4 mL of chloroform and 150 mL of *Diluent*. Shake the mixture on a wrist action shaker for 15 min, and dilute with *Diluent* to 200 mL. Pass the solution through a filter of 0.45- μ m pore size, and discard the first 5 mL of the filtrate.

Blank: Add 340 mg of *Purified Water* to 4 mL of chloroform and 150 mL of *Diluent*. Shake the mixture on a wrist action shaker for 15 min, and dilute with *Diluent* to 200 mL. Pass the solution through a filter of 0.45- μ m pore size, and discard the first 5 mL of the filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Columns

Guard: 4.6-mm \times 2-cm; packing L1

Analytical: 4.6-mm \times 30-cm; 10- μ m packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[NOTE—The retention time for piroxicam is about 7 min.]

Suitability requirements

Relative standard deviation: NMT 2.8% for replicate injections

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Piroxicam RS in the *Standard solution* (μ g/mL)

C_U = nominal concentration of piroxicam in the *Sample solution* (μ g/mL)

Acceptance criteria: 90.0%–110.0%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Package in a tight, light-resistant, plastic resealable container. Store at controlled room temperature.

• BEYOND-USE DATE: NMT 90 days after the date on which it was compounded when stored at controlled room temperature

• LABELING: Label it to state the *Beyond-Use Date*.

• USP REFERENCE STANDARDS (11)

USP Piroxicam RS

Piroxicam Compounded Oral Suspension**DEFINITION**

Piroxicam Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$).

Prepare Piroxicam Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Piroxicam powder	1 g
Ora-Blend, ^a a sufficient quantity to make	100 mL

^a Perrigo, Minneapolis, MN.

Pour the weighed *Piroxicam powder* into a suitable mortar. Wet the powder with a small amount of *Ora-Blend*, and triturate to make a smooth paste. Add the *Ora-Blend* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated container. Add sufficient *Ora-Blend* to bring the preparation to final volume. Shake to mix well.

ASSAY**• PROCEDURE**

Mobile phase: Mix 500 mL of methanol and 500 mL of 0.1 M sodium acetate, and adjust with phosphoric acid to a pH of 4.0. Add 10 mL of acetonitrile, filter, and degas.

Standard solution: 0.2 mg/mL of piroxicam prepared from USP Piroxicam RS in methanol

Sample solution: Shake thoroughly each bottle of Oral Suspension. Transfer 1.0 mL of the Oral Suspension into a 50-mL volumetric flask, dilute with methanol to volume, and mix well to dissolve. Pass through a PVDF filter of 0.45- μ m pore size, discarding the first 3 mL of filtrate.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 361 nm

Column: 3.9-mm \times 15-cm; 4- μ m packing L7

Flow rate: 1.0 mL/min

Injection volume: 5 μ L

System suitability

Sample: *Standard solution*

[NOTE—The retention time for piroxicam is about 4.0 min.]

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of piroxicam from the *Sample solution*

r_S = peak response of piroxicam from the *Standard solution*

C_s = concentration of piroxicam in the *Standard solution* (mg/mL)

C_u = nominal concentration of piroxicam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

SPECIFIC TESTS

- **PH (791):** 3.7–4.7

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or controlled room temperature
- **USP REFERENCE STANDARDS (11)**
USP Piroxicam RS

Plantago Seed

DEFINITION

Plantago Seed is the cleaned, dried, ripe seed of *Plantago psyllium* auct. or of *Plantago indica* L. (*Plantago arenaria* Waldst. & Kit.), known in commerce as Spanish or French psyllium; or of *Plantago ovata* Forssk., known in commerce as blond psyllium, or Indian plantago seed (Fam. Plantaginaceae).

SPECIFIC TESTS

- **BOTANIC CHARACTERISTICS**

Macroscopic

Unground *Plantago psyllium* auct. seed: Ovate to ovate-elongate, concavo-convex; mostly from 1.3 to 2.7 mm in length, rarely up to 3 mm, and from 600 μ m to 1.1 mm in width. It is light brown to moderate brown, darker along the margin, and very glossy; the convex dorsal surface exhibiting a lighter colored longitudinal area extending nearly the length of the seed and representing the embryo lying beneath the seed coat, and showing a sometimes indistinct transverse groove nearer the broader end. The concave ventral surface has a deep cavity, in the center of the base of which is an oval, yellowish white hilum.

Unground *Plantago indica* seed: Ovate-oblong to elliptical, concavo-convex; from 1.6 to 3 mm in length and from 1 to 1.5 mm in width. Externally it is dark reddish brown to moderate yellowish brown, occasionally somewhat glossy, often dull, rough, and reticulate; the convex dorsal surface having a longitudinal lighter colored area extending lengthwise along the center and beneath the seed coat, and a median transverse groove, dent, or fissure. The ventral surface has a deep concavity, the edges somewhat flattened and frequently forming a sharp indented angle with the base of the cavity, the latter showing a light colored oval hilum.

Unground *Plantago ovata* seed: Broadly elliptical to ovate, boat-shaped, from 2 to 3.5 mm in length and from 1 to 1.5 mm in width. It is pale brown to moderate brown with a dull surface, the convex surface having a small, elongated, glossy brown spot. The concave surface has a deep cavity, in the center of the base of which occurs a hilum covered with a thin membrane.

Odor and taste: All varieties of Plantago Seed are nearly odorless.

Microscopic: Plantago Seed is reniform in median transverse sections. Its seed coat has a colorless epidermis of mucilaginous cells whose radial and outer walls

break down to form layers of mucilage when brought into contact with water, and a reddish brown to yellow pigment layer in the seeds of *Plantago indica* and *Plantago psyllium*, a broad endosperm with thick-walled outer palisade cells, and irregular inner endosperm cells; and a straight embryo extending lengthwise through the center. The endosperm and embryo cells contain fixed oil and aleurone grains, the latter being rounded, oval, pyriform, or irregularly shaped, from 2 to 8 μ m in diameter.

- **WATER ABSORPTION**

Sample: 1 g of Plantago Seed

Analysis: Place the *Sample* in a 25-mL graduated cylinder, add water to the 20-mL mark, and shake the cylinder at intervals during 24 h. Allow the seeds to settle for 12 h, and note the total volume occupied by the swollen seeds.

Acceptance criteria: Seeds occupy the following volumes: *Plantago psyllium*, NLT 14 mL; *Plantago ovata*, NLT 10 mL; *Plantago indica*, NLT 8 mL.

- **ARTICLES OF BOTANICAL ORIGIN, Foreign Organic Matter (561):** NMT 0.50%
- **ARTICLES OF BOTANICAL ORIGIN, Total Ash (561):** NMT 4.0%
- **ARTICLES OF BOTANICAL ORIGIN, Acid-Insoluble Ash (561):** NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, secure against insect attack.

Plasma Protein Fraction

» Plasma Protein Fraction conforms to the regulations of the federal Food and Drug Administration concerning biologics (640.90 to 640.96) (see *Biologics* (1041)). It is a sterile preparation of serum albumin and globulin obtained by fractionating material (source blood, plasma, or serum) from healthy human donors, the source material being tested for the absence of hepatitis B surface antigen. It is made by a process that yields a product having protein components of approved composition and sedimentation coefficient content. Not less than 83 percent of its total protein is albumin and not more than 17 percent of its total protein consists of alpha and beta globulins. Not more than 1 percent of its total protein has the electrophoretic properties of gamma globulin. It is a solution containing, in each 100 mL, 5 g of protein, and it contains not less than 94 percent and not more than 106 percent of the labeled amount. It contains no added antimicrobial agent, but it contains sodium acetyltrypophanate with or without sodium caprylate as a stabilizing agent. It has a sodium content of not less than 130 mEq per L and not more than 160 mEq per L and a potassium content of not more than 2 mEq per L. It has a pH between 6.7 and 7.3, measured in a solution diluted to contain 1 percent of protein with 0.15 M sodium chloride. It meets the requirements of the test for heat stability.

Packaging and storage—Preserve at the temperature indicated on the label.

Expiration date—The minimum expiration date is not later than 5 years after issue from manufacturer's cold storage (5°, 1 year) if labeling recommends storage between 2° and 10°; not later than 3 years after issue from manufacturer's cold storage (5°, 1 year) if labeling recommends storage at temperatures not higher than 30°.

Labeling—Label it to state that it is not to be used if it is turbid and that it is to be used within 4 hours after the container is entered. Label it also to state the osmotic equivalent in terms of plasma and the sodium content.

Podophyllum

» Podophyllum consists of the dried rhizomes and roots of *Podophyllum peltatum* L. (Fam. Berberidaceae). It yields not less than 5.0 percent of podophyllum resin.

Botanic characteristics—

Podophyllum—Consists of nearly cylindrical rhizomes, jointed, compressed or flattened somewhat on upper and lower surfaces, and sometimes branched. It occurs as pieces of rhizome up to 20 cm in length, with internodes from 2 mm to 9 mm in diameter, some of the nodes being somewhat thickened. The rhizome is dusky red to light yellowish brown, longitudinally wrinkled or nearly smooth, with irregular, somewhat V-shaped scars of scale leaves; some of the nodes are annulate, the upper portion having large, circular, depressed stem-scars and buds or stem-bases. On the lower portion there are numerous root-scars or roots, the latter from 2 cm to 7 cm in length and about 2 mm in thickness. The fracture is short and weak, the fractured surface being yellowish orange to pale yellow or grayish white.

Histology—The rhizome shows an outer portion consisting of a brown epidermis, often necrosed, and 1 to 3 layers of brown to olive-brown suberized cells; a cortex about 20 cells in width, consisting chiefly of nearly isodiametric cells, the cells containing single or compound starch grains and resin masses and, in scattered cells of the nodes, rosette aggregates of calcium oxalate; a circle of from 16 to 34 open collateral vascular bundles, separated by rather wide medullary rays, each bundle containing a few lignified vessels, a more or less distinct cambium, and a rather large phloem. The pith is large, the cells being more or less rounded and containing starch grains and reddish brown resin masses. The roots show an epidermal layer of brownish suberized cells and a single row of hypodermal cells; a broad cortex of thin-walled nearly isodiametric cells; a distinct endodermis of tangentially elongated cells having uniformly thickened walls; and a 4- to 7-rayed vascular bundle.

Powdered Podophyllum—It is pale brown to weak yellow and has a slight odor. It contains numerous starch grains, simple or 2- to 6-compound, the individual grains being spheroidal, plano- to angular-convex, or polygonal, up to 20 µm in diameter; occasional rosette aggregates of calcium oxalate, up to 80 µm in diameter; vessels with simple pits or reticulate thickenings; fragments of starch- and resin-bearing parenchyma and reddish brown to yellow cork cells.

Indian podophyllum—*Podophyllum peltatum* is differentiated from *Podophyllum hexandrum* Royle (Indian podophyllum) by the reaction described in the test for *Distinction from resin of Indian podophyllum* under *Podophyllum Resin*.

Acid-insoluble ash (561): not more than 2.0%.

Foreign organic matter (561): not more than 2.0%.

Assay—Place 10 g of Podophyllum, in fine powder, in a 125-mL conical flask, add 35 mL of alcohol, and reflux on a steam bath for 3 hours. Transfer the mixture to a small per-

colator, and percolate slowly with warm alcohol until the percolate measures 95 mL. Cool, add sufficient alcohol to the percolate to make it measure 100.0 mL, and mix. Transfer 10.0 mL of this percolate to a separator, and add 10 mL of chloroform and 10 mL of dilute hydrochloric acid (7 in 500). Shake the mixture, allow it to separate, draw off the alcohol-chloroform layer into a second separator, and wash the acid layer with three 15-mL portions of a mixture of chloroform and alcohol (2:1), adding the washings to the second separator. Add 10 mL of dilute hydrochloric acid (7 in 500) to the combined extract and washings, again shake the mixture, allow it to separate, and draw off the alcohol-chloroform layer into a tared vessel. Wash the acid layer three times with 15-mL portions of the alcohol-chloroform mixture, adding the washings to the tared vessel. Evaporate the combined extracts on a steam bath to approximately 1 mL, add 5 mL of dehydrated alcohol, again evaporate to dryness, and dry the residue at 80° for 4 hours: the weight of this residue is the weight of resin in 1 g of the Podophyllum taken.

Podophyllum Resin

» Podophyllum Resin is the powdered mixture of resins extracted from Podophyllum by percolation with Alcohol and subsequent precipitation from the concentrated percolate upon addition to acidified water. It contains not less than 40.0 percent and not more than 50.0 percent of hexane-insoluble matter. [Caution—Podophyllum Resin is highly irritating to the eye and to mucous membranes in general.]

Packaging and storage—Preserve in tight, light-resistant containers.

Identification—

A: It is soluble in 1 N potassium hydroxide or in 1 N sodium hydroxide, forming a yellow liquid, which gradually becomes darker on standing and from which the resin is precipitated by hydrochloric acid.

B: A hot solution of it deposits most of its solids on cooling, and if the cooled liquid is filtered, the filtrate turns brown upon the addition of a few drops of ferric chloride TS.

Residue on ignition (281): not more than 1.5%.

Distinction from resin of Indian podophyllum—Add 400 mg to 3 mL of 60 percent alcohol, then add 0.5 mL of 1 N potassium hydroxide, shake the mixture gently, and allow to stand for 2 hours: it does not gelatinize.

Hexane-insoluble matter—Transfer about 1 g of Podophyllum Resin, accurately weighed, to a glass-stoppered, 100-mL conical flask, add 30.0 mL of chloroform, insert the stopper tightly, and shake for 30 minutes, using a mechanical wrist-action shaker, or equivalent. Filter with suction through a medium- or fine-porosity, sintered-glass filter, into a small filter flask. Wash the conical flask and the filter with two 5-mL portions of chloroform, adding the washings to the filtrate. Transfer the filtrate with the aid of chloroform to a 50-mL volumetric flask, add chloroform to volume, and mix. Pipet 20 mL of the resulting solution into a 250-mL conical flask containing 160 mL of solvent hexane. Gently swirl, allow to stand for 10 minutes, and transfer the resulting precipitate to a tared, fine-porosity, sintered-glass filter, wash the flask and the precipitate with two 20-mL portions of solvent hexane, dry the precipitate at 70° for 1 hour, and weigh the *Hexane-insoluble matter* so obtained. Multiply by 2.5 to find the amount present in the quantity of Podophyllum Resin taken.

Podophyllum Resin Topical Solution

» Podophyllum Resin Topical Solution is a solution in Alcohol consisting of Podophyllum Resin and an alcoholic extract of Benzoin. It contains, in each 100 mL, not less than 10 g and not more than 13 g of hexane-insoluble matter. [Caution—Podophyllum Resin Topical Solution is highly irritating to the eye and to mucous membranes in general.]

Packaging and storage—Preserve in tight, light-resistant containers.

Identification—

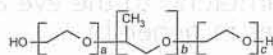
A: A 1 in 5 solution in chloroform is levorotatory.

B: The precipitate obtained as directed in the test for *Hexane-insoluble matter* responds to *Identification test A* under *Podophyllum Resin*.

Alcohol Determination (611): between 69.0% and 72.0% of C_2H_5OH .

Hexane-insoluble matter—Using a 10.0-mL quantity of the Topical Solution, proceed as directed for *Hexane-insoluble matter* under *Podophyllum Resin*. Multiply the weight of hexane-insoluble matter found by 2.5 to find the amount present in the 10.0 mL taken.

Poloxalene



Oxirane, methyl-, polymer with oxirane.

Polyethylene-polypropylene glycol [9003-11-6].

» Poloxalene is a synthetic block copolymer of ethylene oxide and propylene oxide. It contains not less than 98.0 percent and not more than 103.0 percent of poloxalene.

Packaging and storage—Preserve in tight containers, protected from light. Store in a cool place.

USP Reference standards (11)—

USP Poloxalene RS

Labeling—Label it to indicate that it is for veterinary use only.

Identification—

Color reagent—Dissolve 12.7 g of ammonium thiocyanate and 2.0 g of cobalt nitrate in 100 mL of water.

Procedure—Add 10 mL of ethylene chloride to 0.5 g of Poloxalene, and shake for 1 minute. Add 1 mL of *Color reagent*, and shake for 1 minute: a blue color is produced in the lower layer.

Average molecular weight—

Phthalic anhydride-pyridine solution—Prepare as directed in the test for *Average molecular weight* under *Poloxamer*.

Procedure—Using about 12 g of Poloxalene, accurately weighed, proceed as directed for *Procedure* in the test for *Average molecular weight* under *Poloxamer*: the average molecular weight is between 2850 and 3150.

pH (791): between 5.0 and 7.5, in a solution (1 in 40).

Water Determination, Method Ia (921): not more than 0.4%.

Hydroxyl value (401)—

Esterification reagent—Dissolve 166 g of phthalic anhydride and 28 g of imidazole in 1000 mL of pyridine, and allow to stand for 2 hours before using. Store in a light-resistant bottle.

Procedure—Transfer a quantity of Poloxalene, determined by dividing 420 by the expected hydroxyl value and accurately weighed, to a glass-stoppered, 250-mL conical flask, and add 25.0 mL of *Esterification reagent*. Transfer 25.0 mL of *Esterification reagent* to a second glass-stoppered, 250-mL conical flask to provide the reagent blank. Add glass beads to the flasks, swirl to dissolve the Poloxalene, and fit both flasks with glass-jointed reflux condensers previously rinsed with 10 mL of pyridine, and heat on a steam bath for 15 minutes. Add 10 mL of pyridine through each condenser, insert the stoppers, and cool under running water for 1 minute. After removing the condensers, add 10 mL of water and 1 mL of phenolphthalein TS, and titrate with 0.5 N sodium hydroxide VS to a light pink endpoint that persists for at least 15 seconds. Calculate the hydroxyl value by the formula:

$$(56.11N / W)(B - U)$$

in which N is the normality of the 0.5 N sodium hydroxide titrant; W is the weight, in g, of the Poloxalene taken; B and U are the volumes, in mL, of 0.5 N sodium hydroxide consumed by the reagent blank and the solution of Poloxalene, respectively. [NOTE—If B minus U is greater than 10 mL, repeat the test using a smaller sample.] The hydroxyl value is between 36.0 and 40.0.

Cloud point—Add 10 g of Poloxalene to 190.0 mL of water in a 250-mL beaker. Add a magnetic stirring bar, place on a stirrer and hot plate, and stir until dissolution is complete. Place a probe from an electronic thermometer with an accuracy of 0.2° in the solution within 3 mm of the stirring bar. Continue stirring at a rate that minimizes the formation of bubbles. Adjust the hot plate so that the temperature of the solution increases at a rate of about 1° per minute. Continue to view the solution, and record the temperature when the probe can no longer be seen. This occurs between 42.5° and 46.5° .

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Poloxalene RS in ethylene dichloride to obtain a solution having a known concentration of about 0.1575 mg per mL.

Assay preparation—Transfer about 105 mg of Poloxalene, accurately weighed, to a 100-mL volumetric flask, add about 85 mL of ethylene dichloride, and swirl to dissolve. Dilute with ethylene dichloride to volume, and mix. Transfer 15.0 mL of this solution to a second 100-mL volumetric flask, dilute with ethylene dichloride to volume, and mix.

Procedure—Transfer 10.0 mL of the *Standard preparation*, the *Assay preparation*, and ethylene dichloride (to serve as a blank) to glass-stoppered tubes, add 4.0 mL of the *Color reagent* specified in the *Identification* test, shake vigorously for 3 minutes, and then centrifuge for 5 minutes. Carefully remove the lower ethylene dichloride layers from the three tubes, and using the ethylene dichloride layer from the tube containing the blank to zero the spectrophotometer, determine the UV absorbance at 630 nm of the solutions from the *Standard preparation* and the *Assay preparation*. Calculate the percentage of poloxalene in the portion of Poloxalene taken by the formula:

$$100(A_U / A_S)(C_S / C_U)$$

in which A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively; and C_S and C_U are the concentrations, in mg per mL, of the *Standard preparation* and the *Assay preparation*, respectively.

Polycarbophil

Polycarbophil.
Polycarbophil [9003-97-8].

» Polycarbophil is polyacrylic acid cross-linked with divinyl glycol.

Packaging and storage—Preserve in tight containers.

Identification—

A: A dispersion (1 in 100) is orange with thymol blue TS and yellow with cresol red TS.

B: Adjust a dispersion (1 in 100) with 1 M sodium hydroxide to a pH of about 7.5. A very viscous gel is produced.

pH (791)—To 1.0 g add 100 mL of water, and shake by mechanical means for 1 hour: the pH of the mixture is not more than 4.0.

Loss on drying (731)—Dry it in vacuum at 45° for 4 hours: it loses not more than 1.5% of its weight.

Residue on ignition (281): not more than 4.0%.

Absorbing power—Transfer about 50 mg, accurately weighed, to an accurately tared 50-mL centrifuge tube fitted with a tight closure. Add 35 mL of sodium bicarbonate solution (1.5 in 100), and shake manually, venting as necessary to release liberated carbon dioxide. Shake and vent at least 3 times. Close the tube tightly, and shake vigorously by mechanical means for 60 minutes. Centrifuge at 2000 rpm for 1 hour. By means of a 50-mL syringe fitted with a 13-gauge needle, draw off the supernatant taking care that the solid material is not disturbed. Repeat the process of the addition of 35 mL of sodium bicarbonate solution, of the shaking, and of the withdrawing of supernatant. Accurately weigh the tube with its contents, and calculate the weight of the absorbed solution by subtracting the weight of Polycarbophil taken and the tare weight of the tube. The absorbed weight is not less than 62 g per g of Polycarbophil on the dried basis.

Limit of acrylic acid—

pH 3.0 phosphate buffer—Prepare 0.01 M monobasic potassium phosphate, and adjust with phosphoric acid to a pH of 3.0 ± 0.5.

Mobile phase—Prepare a degassed solution consisting of pH 3.0 phosphate buffer and methanol (8:2).

Standard solution—Dissolve an accurately weighed quantity of acrylic acid in water to obtain a solution containing 1.0 mg per mL. Dilute quantitatively, and stepwise if necessary, with a 2.5% alum solution to obtain a solution having a known concentration of about 30 µg per mL.

Test solution—Transfer about 0.1 g of Polycarbophil into a vial. Add 20 mL of a 2.5% alum solution and mix. Heat at 50° for 20 minutes, then shake for 1 hour, centrifuge, and filter.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard solution*, and record peak responses as directed for *Procedure*: the relative standard deviation for replicate injections of the *Standard solution* is not more than 5.0%, and the tailing factor is not more than 2.5.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the response for the acrylic acid peak. Calculate the percentage of

acrylic acid in the portion of Polycarbophil taken by the formula:

$$(0.002C / W)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of acrylic acid, *W* is the weight, in g, of Polycarbophil taken, and *r_U* and *r_S* are the acrylic acid peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.3% acrylic acid is found.

Limit of ethyl acetate—

Internal standard solution—Dissolve an accurately weighed quantity of methyl ethyl ketone in methanol, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.075 mg per mL.

Standard solution—Dissolve 0.225 mg, accurately weighed, of ethyl acetate into a 10-mL volumetric flask. Add 2.0 mL of the *Internal standard solution*, dilute with methanol to volume, and mix.

Test solution—Transfer about 50 mg of Polycarbophil into a 10-mL volumetric flask. Add 2.0 mL of the *Internal standard solution*, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 10-ft. × 2-mm column that contains 1% liquid phase G25 on support S12. The column is maintained at about 160°, and the injection port and detector block are maintained at about 250°. The carrier gas is dry helium flowing at a rate of about 30 mL per minute. Chromatograph the *Standard solution*, and record the chromatogram as directed for *Procedure*: the relative retention times are about 1.3 for ethyl acetate, and 1.0 for methyl ethyl ketone, and the relative standard deviation for replicate injections of the ethyl acetate peak is not more than 2%.

Procedure—Separately inject equal volumes (about 2 µL) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of ethyl acetate in the portion of Polycarbophil taken by the formula:

$$(0.001C / W)(R_U / R_S)$$

in which *C* is the concentration of ethyl acetate, in µg per mL, *W* is the weight of Polycarbophil, in g, and *R_U* and *R_S* are the ratios of the responses of the ethyl acetate peak to the methyl ethyl ketone peak obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.45% is found.

Polyethylene Glycol 3350

Poly(oxy-1,2-ethanediyl), α-hydro-ω-hydroxy-; 1,2-Ethanediol, homopolymer [25322-68-3].

DEFINITION

Polyethylene Glycol 3350 is an addition polymer of ethylene oxide and water, represented by the formula $H(OCH_2CH_2)_nOH$, in which *n* represents the average number of oxyethylene groups. The apparent weight-average molecular weight is 3015–3685 g/mol (Da). It contains NLT 97.0% and NMT 103.0% of polyethylene glycol 3350, calculated on the anhydrous basis. It may contain a suitable antioxidant.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197F) or (197A): Use a thin film of test specimen, melted if necessary, in the range from 4000 to 650 cm⁻¹, when the measurement is performed by using (197F).

B. CHROMATOGRAPHIC IDENTITY

Analysis: Proceed as directed in the Assay.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

PROCEDURE

Mobile phase: 50 µg/mL of sodium azide in water

Standard solution: 20 mg/mL of USP Polyethylene Glycol 3350 RS in *Mobile phase*

Sample solution: 20 mg/mL of Polyethylene Glycol 3350 in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Differential refractive index

Columns

Guard: 6-mm × 4-cm; 6-µm packing L25

Analytical: 7.8-mm × 30-cm; 6-µm packing L25

Temperatures

Detector: 35 ± 1°

Column: 35 ± 1°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for polyethylene glycol 3350 is about 8.5 min.]

Suitability requirements

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of polyethylene glycol 3350 [$\text{H}(\text{OCH}_2\text{CH}_2)_n\text{OH}$] in the portion of Polyethylene Glycol 3350 taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*

C_s = concentration of USP Polyethylene Glycol 3350 RS in the *Standard solution* (mg/mL)

C_u = concentration of Polyethylene Glycol 3350 in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

RESIDUE ON IGNITION (281)

Sample: 2 g

Analysis: Proceed as directed, moistening the residue with 2 mL of sulfuric acid.

Acceptance criteria: NMT 0.1%

Delete the following:

HEAVY METALS (231)

Test preparation: 4.0 g in 5.0 mL of 0.1 N hydrochloric acid. Dilute with water to 25 mL.

Acceptance criteria: NMT 5 µg/g (Official 1-Jan-2018)

LIMIT OF ETHYLENE OXIDE AND DIOXANE

Analysis: Proceed as directed in *Ethylene Oxide and Dioxane* (228), *Method II*.

Acceptance criteria

Ethylene oxide: NMT 1 µg/g

Dioxane: NMT 10 µg/g

LIMIT OF ETHYLENE GLYCOL AND DIETHYLENE GLYCOL

Mobile phase: 50 µg/mL of sodium azide in water

Eluant: Water

Standard stock solution: 10 mg/g of USP Diethylene Glycol RS and 10 mg/g of USP Ethylene Glycol RS in *Eluant*

Standard solution: Transfer 0.1 g of *Standard stock solution* to a 100-mL volumetric flask. Dilute with water to

volume. The *Standard solution* contains 0.01 mg/mL of USP Diethylene Glycol RS and 0.01 mg/mL of USP Ethylene Glycol RS.

Sample solution: 10 mg/mL of Polyethylene Glycol 3350 in *Eluant*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Differential refractive index

Column: 7.8-mm × 30-cm; 7-µm packing L89, 125-Å pore size

Temperatures

Detector: 35 ± 1°

Column: 35 ± 1°

Flow rate: 0.5 mL/min

Injection volume: 100 µL

Run time: 30 min

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for diethylene glycol and ethylene glycol are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 0.9 between diethylene glycol and ethylene glycol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of diethylene glycol (or ethylene glycol) in the portion of Polyethylene Glycol 3350 taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of diethylene glycol (or ethylene glycol) from the *Sample solution*

r_s = peak response of diethylene glycol (or ethylene glycol) from the *Standard solution*

C_s = concentration of USP Diethylene Glycol RS (or USP Ethylene Glycol RS) in the *Standard solution* (mg/mL)

C_u = concentration of Polyethylene Glycol 3350 in the *Sample solution* (mg/mL)

Acceptance criteria

Ethylene glycol: NMT 0.062%

Sum of diethylene glycol and ethylene glycol: NMT 0.2%

LIMIT OF FORMALDEHYDE AND ACETALDEHYDE

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time ^a (min)	Solution A (%)	Solution B (%)
0	50	50
11	0	100

^a The equilibration time is 5 min.

[NOTE—Use amber containers and amber autosampler vials.]

2,4-DNPH solution: Transfer 250 mg of 2,4-dinitrophenylhydrazine (2,4-DNPH) to a 50-mL volumetric flask, add 20.0 mL of acetonitrile, and swirl to mix. Add 3.0 mL of hydrochloric acid to the flask, and swirl to mix. Sonicate until all solids are dissolved, and dilute with acetonitrile to volume.

Formaldehyde-2,4-DNPH solution: 100 µg/mL of aldehyde equivalent in acetonitrile¹

Acetaldehyde-2,4-DNPH solution: 1000 µg/mL of aldehyde equivalent in acetonitrile²

Formaldehyde stock solution: Transfer 500 µL of *Formaldehyde-2,4-DNPH solution* to a 10-mL volumetric

¹ Available from Sigma-Aldrich Corporation, or equivalent.

² Available from Sigma-Aldrich Corporation, or equivalent.

flask. Dilute with acetonitrile to volume. The formaldehyde concentration is 5.0 µg/mL.

Acetaldehyde stock solution: Transfer 500 µL of *Acetaldehyde-2,4-DNPH solution* to a 10-mL volumetric flask. Dilute with acetonitrile to volume. The acetaldehyde concentration is 50.0 µg/mL.

Standard solution: Transfer 1.5 mL of *Formaldehyde stock solution* and 1.2 mL of *Acetaldehyde stock solution* to a 10-mL volumetric flask. Dilute with acetonitrile to volume, and mix well. The concentrations of formaldehyde and acetaldehyde are 0.75 and 6.0 µg/mL, respectively.

Sample solution: Transfer 0.5 g of Polyethylene Glycol 3350 to a 10-mL volumetric flask. Add 1.0 mL of acetonitrile to the flask, and swirl to dissolve the sample. Add 2.0 mL of *2,4-DNPH solution* to the flask, and swirl to mix. Allow the solution to react for 15 min, then dilute with acetonitrile to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 360 nm

Column: 3.0-mm × 15-cm; 3.5-µm packing L7, 80-Å pore size

Column temperature: 30 ± 1°

Flow rate: 0.65 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for formaldehyde and acetaldehyde are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 2.0 between formaldehyde and acetaldehyde

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the content of formaldehyde (or acetaldehyde), in µg/g, in the portion of Polyethylene Glycol 3350 taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u)$$

r_u = peak response of formaldehyde (or acetaldehyde) from the *Sample solution*

r_s = peak response of formaldehyde (or acetaldehyde) from the *Standard solution*

C_s = concentration of formaldehyde (or acetaldehyde) in the *Standard solution* (µg/mL)

C_u = concentration of Polyethylene Glycol 3350 in the *Sample solution* (g/mL)

Acceptance criteria

Formaldehyde: NMT 15 µg/g

Sum of formaldehyde and acetaldehyde: NMT 200 µg/g

SPECIFIC TESTS

• APPARENT WEIGHT-AVERAGE MOLECULAR WEIGHT AND POLYDISPERSITY

Mobile phase: Water

Standard solution: 1.0 mg/mL each of five polyethylene glycol standards with molecular weights of 1000, 2000, 3000, 4000, and 6000 g/mol (Da) in *Mobile phase*. Pass a portion of the solution through a 0.45-µm polytetrafluoroethylene (PTFE) syringe filter.³ Discard the first 2 mL, and transfer the solution for analysis.

Sample solution: 1.0 mg/mL of Polyethylene Glycol 3350 in *Mobile phase*. Pass a portion of the solution through a 0.45-µm PTFE syringe filter.³ Discard the first 2 mL, and transfer the solution for analysis.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Differential refractive index

Column: 7.8-mm × 30-cm; 6-µm packing L37

Temperatures

Detector: 35 ± 1°

Column: 35 ± 1°

Flow rate: 0.8 mL/min

Injection volume: 10 µL

Run time: 18 min

Analysis

Samples: *Standard solution* and *Sample solution*

Separately inject equal volumes of the *Standard solution* and the *Sample solution* into the chromatograph, record the chromatograms, and determine the elution peak maxima and the corresponding retention times for the five polyethylene glycol standards.

Calibration curve: Plot the retention times on the x-axis against the log M_p (M_p : peak molecular weights) on the y-axis for the peaks from the polyethylene glycol standard to generate a calibration curve using suitable gel permeation chromatography or size exclusion chromatography (GPC/SEC) software.

Calculations: Compute the data using the same GPC/SEC software, and determine the number- and weight-average molecular weights, M_N and M_W , in g/mol (Da), respectively, for the chromatogram of the *Sample solution*.

Calculate the polydispersity for Polyethylene Glycol 3350:

$$\text{Result} = M_W/M_N$$

M_W = weight-average molecular weight from the *Sample solution* (g/mol)

M_N = number-average molecular weight from the *Sample solution* (g/mol)

Acceptance criteria: The value of apparent weight-average molecular weight is 3015–3685 g/mol. Polydispersity is 90%–110% of the value stated on the label or within the range indicated on the label.

• HYDROXYL VALUE

Phthalic anhydride solution: Place 49.0 g of phthalic anhydride into an amber bottle, and dissolve in 300 mL of pyridine from a freshly opened bottle or pyridine that has been freshly distilled over phthalic anhydride. Shake vigorously until completely dissolved. Add 7 g of imidazole, swirl carefully to dissolve, and allow to stand for 16 h before using.

Sample solution: Carefully introduce 25.0 mL of *Phthalic anhydride solution* into a dry, heat-resistant pressure bottle. Add 12.0 g of Polyethylene Glycol 3350. Add 25 mL of pyridine, from a freshly opened bottle or pyridine that has been freshly distilled over phthalic anhydride. Swirl to dissolve, insert the stopper in the bottle, and wrap it securely in a cloth bag.

Blank: 25.0 mL of *Phthalic anhydride solution* plus any additional pyridine added to the bottle

Analysis: Immerse the bottle in a water bath maintained at a temperature between 96° and 100°, to the same depth as that of the mixture in the bottle. Remove the bottles from the bath after 5 min and, without unwrapping, swirl for 30 s to homogenize. Heat in the water bath for 60 min, then remove from the bath, and allow it to cool to room temperature. Uncap the bottle carefully to release any pressure, remove from the bag, add 10 mL of water, and swirl thoroughly. Wait 2 min, add 0.5 mL of a solution of phenolphthalein in pyridine (1 in 100), and titrate with 0.5 N sodium hydroxide VS to the first pink color that persists for 15 s. Perform a blank determination.

Calculate the hydroxyl value:

$$\text{Result} = [M_r \times (V_B - V_s) \times N]/W$$

³ Millipore® Millex® LCR HPLC syringe filters with hydrophilic PTFE membrane is suitable, or any other equivalent filter.

- M_r = molecular weight of potassium hydroxide, 56.11
 V_B = volume of 0.5 N sodium hydroxide consumed in the blank test (mL)
 V_S = volume of 0.5 N sodium hydroxide consumed in the actual test (mL)
 N = exact normality of the sodium hydroxide solution
 W = weight of Polyethylene Glycol 3350 taken for the test (g)

Acceptance criteria: 30–38

• ACIDITY AND ALKALINITY

Sample solution: Dissolve 5.0 g of Polyethylene Glycol 3350 in 100 mL of carbon dioxide-free water.

Analysis: Add 0.3 mL of a saturated solution of potassium chloride into the *Sample solution*. The test solution should be maintained at $25 \pm 2^\circ$ during the measurement. Measure the pH following pH (791).

Acceptance criteria: 4.5–7.5

• WATER DETERMINATION (921), Method I

Sample: 2.0 g

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from direct sunlight. Avoid exposure to excessive heat.
- **LABELING:** Label it to indicate its polydispersity (M_w/M_n) or its polydispersity range. Label it to indicate the name and amount of any added antioxidant.
- **USP REFERENCE STANDARDS (11)**
 USP Diethylene Glycol RS
 USP Ethylene Glycol RS
 USP Polyethylene Glycol 3350 RS

Polyethylene Glycol 3350 and Electrolytes for Oral Solution

(Prior to August 1, 2012, the current practice of labeling the article of commerce with the name PEG 3350 and Electrolytes for Oral Solution may be continued. Use of the name Polyethylene Glycol 3350 and Electrolytes for Oral Solution will be permitted as of August 1, 2007, but the use of this name will not be mandatory until August 12, 2012. The 60-month extension will provide the time needed by the manufacturers and users to make necessary changes.)

» Polyethylene Glycol 3350 and Electrolytes for Oral Solution is a mixture of Polyethylene Glycol 3350, Sodium Bicarbonate, Sodium Chloride, Sodium Sulfate (anhydrous), and Potassium Chloride. When constituted as directed in the labeling it contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of polyethylene glycol 3350, potassium (K^+), sodium (Na^+), bicarbonate (HCO_3^-), chloride (Cl^-), and sulfate (SO_4^{2-}), the labeled amounts per L being 10 mmol (10 mEq) of potassium, 125 mmol (125 mEq) of sodium, 20 mmol (20 mEq) of bicarbonate, 35 mmol (35 mEq) of chloride, and 40 mmol (80 mEq) of sulfate.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—

USP Polyethylene Glycol 3350 RS

Completeness of solution (641): meets the requirements.

Identification—

A: The IR absorption spectrum of a mineral oil dispersion of it in a calcium fluoride cell exhibits maxima only at the same wavelengths as that of a similar preparation of USP Polyethylene Glycol 3350 RS.

B: A solution (1 in 20) responds to the tests for *Sodium* (191), *Potassium* (191), *Bicarbonate* (191), *Sulfate* (191), and *Chloride* (191).

pH (791): between 7.5 and 9.5, in the solution prepared as directed in the labeling.

Uniformity of dosage units (905): meets the requirements.

Osmolality and Osmolarity (785), Osmolality: between 235 and 304 mOsmol, in the solution prepared as directed in the labeling.

Assay for potassium and sodium—

Mobile phase—Dilute 0.5 mL of nitric acid with water to obtain 4000 mL of solution. Degas, and place the solution in a suitable plastic container. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Internal standard solution—Dissolve a suitable quantity of ammonium bromide in water to obtain a solution having a concentration of about 2 mg per mL.

Standard preparation—To a 100-mL volumetric flask transfer about 90 mg of potassium chloride, previously dried at 105° for 2 hours and accurately weighed, and about 880 mg of sodium chloride, previously dried at 105° for 2 hours and accurately weighed, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with water to volume, and mix. Pass this solution through a filter having a 0.5- μ m or finer porosity, and store the filtrate in a suitable plastic container. This *Standard preparation* contains about 9 μ g (0.00012 mEq) of potassium chloride and about 88 μ g (0.0015 mEq) of sodium chloride per mL.

Assay preparation—Constitute the contents of a container of Polyethylene Glycol 3350 and Electrolytes for Oral Solution with an accurately measured volume of water, as specified in the labeling. Transfer 6.0 mL of this stock solution, equivalent to about 0.06 mEq of potassium, to a 500-mL volumetric flask, add 10 mL of *Internal standard solution*, dilute with water to volume, and mix. This solution contains about 0.00012 mEq of potassium and 0.0015 mEq of sodium per mL. [NOTE—Reserve the remaining portion of the stock solution for the *Assay for bicarbonate*, and reserve the remaining portion of the *Assay preparation* for the *Assay for chloride and sulfate* and the *Assay for polyethylene glycol 3350*.]

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a conductivity detector, a 4-mm \times 5-cm guard column containing packing L17, and a 4-mm \times 30-cm analytical column maintained at $35 \pm 1^\circ$ containing packing L17. The flow rate is about 0.9 mL per minute. Chromatograph the *Standard preparation* as directed for *Procedure*: the relative retention times are about 0.6 for sodium, 0.8 for ammonium, and 1.0 for potassium; the resolution, R , between the sodium and ammonium peaks is not less than 1.1, and between the ammonium and potassium peaks is not less than 0.9. [NOTE—Maintain column backpressure at less than 1000 pounds per square inch. Backpressure may be reduced by changing the in-line filters and frits in the columns. Column efficiency may be improved by backflushing the analytical column with 30 mL of 0.1 N nitric acid or by injecting four successive 100- μ L portions of 0.1 N nitric acid into the chromatograph.]

Procedure—[NOTE—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Cal-

culate the mEq of potassium per L of constituted Oral Solution taken by the formula:

$$(500 / 74.55)(C / 6)(R_U / R_S)$$

in which 74.55 is the molecular weight of potassium chloride; C is the concentration, in μg per mL, of potassium chloride in the *Standard preparation*; and R_U and R_S are the peak response ratios of potassium to ammonium obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the mEq of sodium per L of constituted Oral Solution taken by the formula:

$$(500 / 58.44)(C / 6)(R_U / R_S)$$

in which 58.44 is the molecular weight of sodium chloride; C is the concentration, in μg per mL, of sodium chloride in the *Standard preparation*; and R_U and R_S are the peak response ratios of sodium to ammonium obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for bicarbonate—Transfer 400.0 mL of the stock solution remaining from the *Assay for potassium and sodium*, equivalent to about 672 mg of sodium bicarbonate (8 mEq), to a suitable container, add methyl red TS, and titrate with 1 N sulfuric acid VS. Calculate the mEq of bicarbonate (HCO_3^-) per L of constituted Oral Solution taken by the formula:

$$2.5V_A$$

in which V_A is the volume, in mL, of 1 N sulfuric acid consumed.

Assay for chloride and sulfate—

Mobile phase—Transfer 34 g of boric acid, 8.6 g of lithium hydroxide, 23.5 mL of gluconic acid solution (1:1), and 125 mL of glycerin to a 1000-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. Add 15 mL of this buffer solution to 865 mL of water, mix, and degas. Add 120 mL of acetonitrile, mix, and degas. [NOTE—Protect the *Mobile phase* from air to prevent absorption of carbon dioxide.] Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Increasing the proportion of buffer solution decreases the retention times of the analytes.

Internal standard solution—Dissolve a suitable quantity of ammonium bromide in water to obtain a solution having a concentration of about 2.2 mg per mL.

Standard preparation—To a 100-mL volumetric flask transfer about 246 mg of sodium chloride (4.2 mEq), previously dried at 105° for 2 hours and accurately weighed, and about 682 mg of anhydrous sodium sulfate (9.6 mEq), previously dried at 105° for 2 hours and accurately weighed, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with water to volume, and mix. Filter this solution through a 0.5- μm or finer porosity filter, and store the filtrate in a suitable glass container. This *Standard preparation* contains about 24.6 μg of sodium chloride (0.00042 mEq of chloride) and about 68.2 μg of sodium sulfate (0.00096 mEq of sulfate) per mL.

Assay preparation—Use the *Assay preparation* prepared as directed in the *Assay for potassium and sodium*. This solution contains about 0.042 mEq of chloride and 0.096 mEq of sulfate per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a conductivity detector, a 4-mm \times 5-cm guard column containing packing L23, and a 4-mm \times 30-cm analytical column maintained at $35 \pm 1^\circ$ containing packing L23. The flow rate is about 0.9 mL per minute. Chromatograph the *Standard preparation* as directed for *Procedure*: the relative retention times are about 0.25 for chloride, 0.4 for bromide, and 1.0 for sulfate, the resolution, R , between the chloride and bromide peaks

is not less than 1.5 and between the bromide and sulfate peaks is not less than 4.5. [NOTE—Maintain column backpressure at less than 1000 pounds per square inch. Backpressure may be reduced by changing the in-line filters and frits in the columns. Column efficiency may be improved by backflushing the analytical column with 50 mL of the buffer solution used to prepare the *Mobile phase*.]

Procedure—[NOTE—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the mEq of chloride per L of constituted Oral Solution taken by the formula:

$$(500 / 58.44)(C / 6)(R_U / R_S)$$

in which 58.44 is the molecular weight of sodium chloride, C is the concentration, in μg per mL, of sodium chloride in the *Standard preparation*, and R_U and R_S are the peak response ratios of chloride to bromide obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the mEq of sulfate per L of constituted Oral Solution taken by the formula:

$$(500 / 71.02)(C / 6)(R_U / R_S)$$

in which 71.02 is one-half of the molecular weight of sodium sulfate, C is the concentration, in μg per mL, of anhydrous sodium sulfate in the *Standard preparation*, and R_U and R_S are the peak response ratios of sulfate to bromide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Assay for polyethylene glycol 3350—

Salt solution—Prepare a solution in water containing 0.35 mg of sodium chloride, 0.18 mg of potassium chloride, 0.40 mg of sodium bicarbonate, 1.37 mg of anhydrous sodium sulfate, and 0.88 mg of ammonium bromide per mL.

Mobile phase—Dilute 40.0 mL of *Salt solution* with water to 1000 mL. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Transfer about 360 mg of USP Polyethylene Glycol 3350 RS, accurately weighed, to a 500-mL volumetric flask, add 20.0 mL of *Salt solution* and about 250 mL of water, dissolve by swirling, dilute with water to volume, and mix. This *Standard preparation* contains about 0.72 mg of polyethylene glycol 3350 per mL.

Assay preparation—Use the *Assay preparation*, prepared as directed in the *Assay for potassium and sodium*. This solution contains about 0.72 mg of polyethylene glycol 3350 per mL.

Chromatographic system (see *Chromatography* (621))—[NOTE—Use peak heights where peak responses are indicated.] The liquid chromatograph is equipped with a refractive index detector maintained at $34 \pm 0.5^\circ$, a 7.8-mm \times 4.5-cm guard column containing packing L25, and a 7.8-mm \times 30-cm analytical column containing packing L25 and maintained at ambient temperature. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation* as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 1.5%. [NOTE—Maintain column backpressure at less than 1000 pounds per square inch. Backpressure may be reduced by cleaning the frits in the guard column or by replacing the guard column. Baseline drift may be reduced by maintaining strict control of ambient temperature, by insulating the lines, the *Mobile phase* reservoir, and the columns, and by increasing the time of equilibration.]

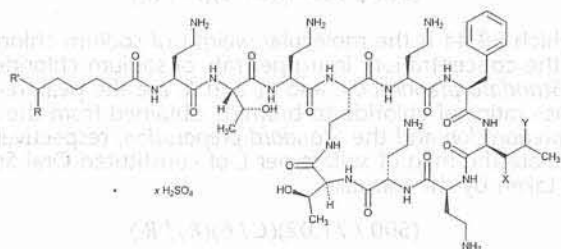
Procedure—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the con-

tent, in g, of polyethylene glycol 3350 per L of constituted Oral Solution taken by the formula:

$$500(C/6)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of polyethylene glycol 3350 in the *Standard preparation*, and r_U and r_S are the polyethylene glycol 3350 peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Polymyxin B Sulfate



Polymyxin	R	R'	X	Y
B1			H	
B2			H	
B3		H	H	
B14				H

$C_{56}H_{98}N_{16}O_{13}$ 1203.48
Polymyxin B1:

$N-[(S)-4\text{-Amino-1-}[(2S,3R)-1-[(S)-4\text{-amino-1-oxo-1-}[(3S,6S,9S,12S,15S,18S,21S)-6,9,18\text{-tris(2-aminoethyl)-15-benzyl-3-}[(R)-1\text{-hydroxyethyl]-12-isobutyl-2,5,8,11,14,17,20-heptaaxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl)amino)butan-2-yl]amino]-3-hydroxy-1-oxobutan-2-yl]amino]-1-oxobutan-2-yl]-6\text{-methyloctanamide}$ [4135-11-9].

$C_{55}H_{96}N_{16}O_{13}$ 1189.45
Polymyxin B2:

$N-[(S)-4\text{-Amino-1-}[(2S,3R)-1-[(S)-4\text{-amino-1-oxo-1-}[(3S,6S,9S,12S,15S,18S,21S)-6,9,18\text{-tris(2-aminoethyl)-15-benzyl-3-}[(R)-1\text{-hydroxyethyl]-12-isobutyl-2,5,8,11,14,17,20-heptaaxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl)amino)butan-2-yl]amino]-3-hydroxy-1-oxobutan-2-yl]amino]-1-oxobutan-2-yl]-6\text{-methylheptanamide}$ [34503-87-2].

$C_{55}H_{96}N_{16}O_{13}$ 1189.45
Polymyxin B3:

$N-[(S)-4\text{-Amino-1-}[(2S,3R)-1-[(S)-4\text{-amino-1-oxo-1-}[(3S,6S,9S,12S,15S,18S,21S)-6,9,18\text{-tris(2-aminoethyl)-15-benzyl-3-}[(R)-1\text{-hydroxyethyl]-12-isobutyl-2,5,8,11,14,17,20-heptaaxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl)amino)butan-2-yl]amino]-3-hydroxy-1-oxobutan-2-yl]amino]-1-oxobutan-2-yl]octanamide$ [71140-58-4].

$C_{56}H_{98}N_{16}O_{13}$ 1203.48
Polymyxin B1-l:

$N-[(S)-4\text{-Amino-1-}[(2S,3R)-1-[(S)-4\text{-amino-1-oxo-1-}[(3S,6S,9S,12S,15S,18S,21S)-6,9,18\text{-tris(2-aminoethyl)-15-benzyl-3-}[(R)-1\text{-hydroxyethyl]-12-}[(S)\text{-sec-butyl]-2,5,8,11,14,17,20-heptaaxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl)amino)butan-2-yl]amino]-3-hydroxy-1-oxobutan-2-yl]amino]-1-oxobutan-2-yl]-6\text{-methyloctanamide}$.

Polymyxin B, sulfate;

Polymyxin B sulfate [1405-20-5].

Polymyxin B [1404-26-8].

DEFINITION

Polymyxin B Sulfate is the sulfate salt of a kind of polymyxin, a substance produced by the growth of *Bacillus polymyxa* (Prazmowski) Migula (Fam. Bacillaceae), or a mixture of two or more such salts. It has a potency of NLT 6000 Polymyxin B units/mg, calculated on the dried basis.

IDENTIFICATION

- A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the test for *Composition of Polymyxins*.
- B.**

Solution A: 10 mg/mL of cupric sulfate

Sample solution: Dissolve 2 mg in 5 mL of water.

Analysis: To the *Sample solution* add 5 mL of 2.5 N sodium hydroxide, and mix. Add 5 drops of *Solution A*, shaking after the addition of each drop.

Acceptance criteria: A reddish-violet color is produced.
- C. IDENTIFICATION TESTS—GENERAL (191), Sulfate:** A 50-mg/mL solution of Polymyxin B Sulfate meets the requirements.

ASSAY

- PROCEDURE**

Analysis: Proceed with Polymyxin B Sulfate as directed for *Antibiotics—Microbial Assays* (81).

Acceptance criteria: NLT 6000 Polymyxin B units/mg on the dried basis

IMPURITIES

- RESIDUE ON IGNITION (281)**

Analysis: Proceed as directed in the chapter, moistening the charred residue with 2 mL of nitric acid and 5 drops of sulfuric acid.

Acceptance criteria: NMT 5.0% where used for prescription compounding

Change to read:

ORGANIC IMPURITIES

Buffer, Mobile phase, Diluent, Standard solution,
 Δ_{USP40} **Sample solution, Chromatographic system,**
 Δ and **System suitability:** Δ_{USP40} Proceed as directed in the test for *Composition of Polymyxins*.

Δ_{USP40}

Analysis

Samples: Δ *Standard solution* (the *Standard solution* is used to determine the disregard limit) and Δ_{USP40} *Sample solution*

Calculate the percentage of each impurity in the portion of Polymyxin B Sulfate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_T = sum of the responses of all peaks from the *Sample solution*

Acceptance criteria: Disregard any peak less than $\Delta 0.007$ times the peak response of the polymyxin B1 peak from the *Standard solution*. Δ_{USP40}

Any individual impurity: NMT 3.0%

Total impurities: NMT 17.0%

SPECIFIC TESTS

PH (791)

Sample solution: 5 mg/mL

Acceptance criteria: 5.0–7.5

LOSS ON DRYING (731)

Analysis: Dry 100 mg in a capillary-stoppered bottle under vacuum at 60° for 3 h.

Acceptance criteria: NMT 7.0%

- STERILITY TESTS (71):** Meets the requirements where the label states that Polymyxin B Sulfate is sterile

• **PYROGEN TEST (151)**

Sample solution: Nominally 20×10^3 Polymyxin B units/mL in pyrogen-free saline TS

Analysis: Proceed as directed in the chapter using a test dose of 1.0 mL/kg.

Acceptance criteria: Meets the requirements where intended for injectable dosage forms or where the label states that Polymyxin B Sulfate must be subjected to further processing during the preparation of injectable dosage forms

Change to read:

• **COMPOSITION OF POLYMYXINS**

Buffer: 4.5 g/L of sodium sulfate anhydrous. The pH is adjusted to 2.3 with dilute phosphoric acid (10% w/w) prior to final dilution.

Mobile phase: Acetonitrile and Buffer (20:80)

Diluent: Acetonitrile and water (20:80)

Standard solution: 0.5 mg/mL of USP Polymyxin B Sulfate RS in Diluent

[▲]USP40

Sample solution: 0.5 mg/mL of Polymyxin B Sulfate in Diluent

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm \times 15-cm; 3.5- μ m packing L1.

[▲][NOTE—A 4.6-mm \times 25-cm; 5- μ m packing L1 column was also found to be suitable.][▲]USP40

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μ L

Run time: 1.4 times the retention time of polymyxin B1

System suitability

Sample: Standard solution

Suitability requirements

Resolution: NLT 3.0 between polymyxin B2 and polymyxin B3

Relative standard deviation: NMT 2.0% for the polymyxin B1 peak

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of polymyxin B1 in the portion of Polymyxin B Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of polymyxin B1 from the Sample solution

r_S = peak response of polymyxin B1 from the Standard solution

C_S = concentration of USP Polymyxin B Sulfate RS in the Standard solution (mg/mL)

C_U = concentration of Polymyxin B Sulfate in the Sample solution corrected for loss on drying (mg/mL)

P = potency of polymyxin B1 in USP Polymyxin B Sulfate RS (mg/mg)

Calculate the percentage of polymyxin B2 in the portion of Polymyxin B Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of polymyxin B2 from the Sample solution

r_S = peak response of polymyxin B2 from the Standard solution

C_S = concentration of USP Polymyxin B Sulfate RS in the Standard solution (mg/mL)

C_U = concentration of Polymyxin B Sulfate in the Sample solution corrected for loss on drying (mg/mL)

P = potency of polymyxin B2 in USP Polymyxin B Sulfate RS (mg/mg)

Calculate the percentage of polymyxin B3 in the portion of Polymyxin B Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of polymyxin B3 from the Sample solution

r_S = peak response of polymyxin B3 from the Standard solution

C_S = concentration of USP Polymyxin B Sulfate RS in the Standard solution (mg/mL)

C_U = concentration of Polymyxin B Sulfate in the Sample solution corrected for loss on drying (mg/mL)

P = potency of polymyxin B3 in USP Polymyxin B Sulfate RS (mg/mg)

Calculate the percentage of polymyxin B1-I in the portion of Polymyxin B Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

r_U = peak response of polymyxin B1-I from the Sample solution

r_S = peak response of polymyxin B1-I from the Standard solution

C_S = concentration of USP Polymyxin B Sulfate RS in the Standard solution (mg/mL)

C_U = concentration of Polymyxin B Sulfate in the Sample solution corrected for loss on drying (mg/mL)

P = potency of polymyxin B1-I in USP Polymyxin B Sulfate RS (mg/mg)

Acceptance criteria: See Table 1. Report the components on the dried basis.

Table 1

Name	Relative Retention Time	Acceptance Criteria, (%)
Polymyxin B2 ^a	0.5	—
Polymyxin B3	0.6	NMT 6.0
Polymyxin B1-I	0.8	NMT 15.0
Polymyxin B1 ^a	1.0	—
Sum of polymyxins B1, B2, B3, and B1-I	—	NLT 80.0

^a These components are not reported individually. They are only reported in the sum of polymyxins.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **LABELING:** Where packaged for prescription compounding, the label states the number of Polymyxin B units in the container and per mg, that it is not intended for manufacturing use, that it is not sterile, and that its potency cannot be assured for longer than 60 days after opening. Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.

- **USP REFERENCE STANDARDS** (11)
USP Polymyxin B Sulfate RS

Polymyxin B for Injection

» Polymyxin B for Injection contains an amount of Polymyxin B Sulfate equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of polymyxin B.

Change to read:

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017), protected from light.

Labeling—Label it to indicate that where it is administered intramuscularly and/or intrathecally, it is to be given only to patients hospitalized so as to provide constant supervision by a physician.

USP Reference standards (11)—
USP Polymyxin B Sulfate RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

Thin-layer chromatographic identification test (201BNP): meets the requirements.

Pyrogen—It meets the requirements of the *Pyrogen Test* (151), the test dose being 1.0 mL per kg of a solution in pyrogen-free saline TS containing 20,000 Polymyxin B Units per mL.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

Particulate Matter in Injections (788): meets the requirements for small-volume injections.

Residue on ignition (281): not more than 5.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

Delete the following:

• **Heavy metals, Method II** (231): not more than 0.01%.
• (Official 1-Jan-2018)

Other requirements—It meets the requirements for *pH and Loss on drying under Polymyxin B Sulfate*. It also meets the requirements for *Uniformity of Dosage Units* (905) and *Labeling* (7), *Labels and Labeling for Injectable Products*.

Change to read:

Assay—

Assay preparation 1 (where it is represented as being in a single-dose container)—Constitute Polymyxin B for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Buffer B.6* (CN 1-May-2017) to obtain a solution containing a convenient number of Polymyxin B Units per mL.

Assay preparation 2 (where the label states the quantity of polymyxin B in a given volume of constituted solution)—Constitute 1 container of Polymyxin B for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accu-

ately measured volume of the constituted solution quantitatively with *Buffer B.6* (CN 1-May-2017) to obtain a solution containing a convenient number of Polymyxin B Units per mL.

Procedure—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer B.6* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Polymyxin B Sulfate and Bacitracin Zinc Topical Aerosol

» Polymyxin B Sulfate and Bacitracin Zinc Topical Aerosol contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of polymyxin B and bacitracin.

Packaging and storage—Preserve in pressurized containers, and avoid exposure to excessive heat.

USP Reference standards (11)—

USP Bacitracin Zinc RS
USP Polymyxin B Sulfate RS

Identification—Collect in a suitable container the expelled contents of 1 Aerosol container, shake with a volume of 0.1 N hydrochloric acid sufficient to obtain a solution containing about 500 USP Bacitracin Units per mL, centrifuge, and use the clear supernatant as the test solution. Proceed as directed under *Thin-Layer Chromatographic Identification Test* (201BNP). The specified result is observed.

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—Collect aseptically in a suitable container half the contents expelled from 5 containers, dissolve in 500 mL of *Fluid A* containing 0.25 g of sodium thioglycollate and adjusted with sodium hydroxide to a pH of 6.6 ± 0.6 , pass through a membrane filter as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined under Sterility Tests* (71), except to place the filter on the surface of Soybean-Casein Digest Agar Medium in a Petri dish, incubate for 7 days at 30° to 35°, and count the number of colonies on the filter. Similarly prepare a second specimen, except to incubate at 20° to 25°. Not more than 20 colonies are observed from the two specimens. It meets also the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* under *Microbial Enumeration Tests* (61) and *Tests for Specified Microorganisms* (62).

Water Determination, Method I (921)—Store 1 container of Topical Aerosol in a freezer for not less than 2 hours, open the container, and transfer 10.0 mL of the freshly mixed specimen to a titration vessel containing 20 mL of a mixture of toluene and methanol (7:3) instead of methanol. In titrating the specimen, determine the endpoint at a temperature of 10° or higher: not more than 0.5% of water is found.

Change to read:

Other requirements—It meets the requirements for *Pressure Test, Minimum Fill, and Leakage Test under Topical Aerosols* (603) (CN 1-May-2017).

Change to read:

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), expel-

ling the entire contents of 1 container of Topical Aerosol, according to the directions in the labeling, into a 2000-mL conical flask held in a horizontal position. Add 500.0 mL of 0.01 N hydrochloric acid, and shake to dissolve. Immediately dilute an accurately measured volume of this acidic solution quantitatively and stepwise with **Buffer B.6** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for bacitracin—Proceed as directed for bacitracin under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of the acidic solution obtained in the *Assay for polymyxin B* immediately diluted quantitatively and stepwise with **Buffer B.1** (CN 1-May-2017) to yield a *Test Dilution* having a bacitracin concentration assumed to be equal to the median dose level of the Standard. [NOTE—Add additional hydrochloric acid to each test dilution of the Standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution*.]

Polymyxin B Sulfate and Bacitracin Zinc Topical Powder

» Polymyxin B Sulfate and Bacitracin Zinc Topical Powder contains not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of polymyxin B and bacitracin.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—

USP Bacitracin Zinc RS

USP Polymyxin B Sulfate RS

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—Collect aseptically in a suitable container about 1 g from not less than 5 containers, dissolve in 500 mL of *Fluid A*, filter through a membrane filter as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined* under *Sterility Tests* (71), except to place the filter on the surface of Soybean-Casein Digest Agar Medium in a Petri dish, incubate for 7 days at 30° to 35°, and count the number of colonies on the filter. Similarly prepare a second specimen, except to incubate at 20° to 25°. Not more than 20 colonies are observed from the two specimens. It meets also the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* under *Microbial Enumeration Tests* (61) and *Tests for specified microorganisms* (62).

Water Determination, Method I (921): not more than 7.0%.

Change to read:

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Topical Powder, equivalent to about 5000 USP Polymyxin B Units, shaken with 20 mL of water in a suitable volumetric flask. Dilute with **Buffer B.6** (CN 1-May-2017) to volume, and mix. Dilute an accurately measured volume of the solution so obtained quantitatively with **Buffer B.6** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.

Change to read:

Assay for bacitracin—Proceed as directed for bacitracin under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Topical Powder, equivalent to about 800 USP Bacitracin Units, added to a 100-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Dilute this solution quantitatively with **Buffer B.1** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. In preparing each test dilution of the Standard, add additional hydrochloric acid to each to obtain the same concentration of hydrochloric acid as in the *Test Dilution*.

Polymyxin B Sulfate and Hydrocortisone Otic Solution

» Polymyxin B Sulfate and Hydrocortisone Otic Solution is a sterile solution containing not less than 90.0 percent and not more than 130.0 percent of the labeled amount of polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone (C₂₁H₃₀O₅). It may contain one or more suitable buffers and preservatives.

NOTE—Where Polymyxin B Sulfate and Hydrocortisone Otic Solution is prescribed without reference to the quantity of polymyxin B or hydrocortisone contained therein, a product containing 10,000 Polymyxin B Units and 5 mg of hydrocortisone per mL shall be dispensed.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—

USP Hydrocortisone RS

USP Polymyxin B Sulfate RS

Sterility Tests (71): meets the requirements.

pH (791): between 3.0 and 5.0.

Change to read:

Assay for polymyxin—Proceed with Otic Solution as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Otic Solution diluted quantitatively with **Buffer B.6** (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for hydrocortisone—

Mobile phase—Prepare a suitable solution of about 500 volumes of methanol, 500 volumes of water, and 1 volume of glacial acetic acid, such that the retention time of hydrocortisone is between 6 and 10 minutes.

Standard preparation—Dissolve a suitable quantity of USP Hydrocortisone RS, accurately weighed, in a mixture of methanol and water (1:1) to obtain a solution having a known concentration of about 0.15 mg per mL.

Assay preparation—Transfer an accurately measured volume of Otic Solution, equivalent to about 15 mg of hydrocortisone, to a 100-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph five replicate

injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, adjusting the specimen size and other operating parameters such that the peak obtained from the *Standard preparation* is about 0.6 full-scale. Record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{21}H_{30}O_5$ in each mL of the Otic Solution taken by the formula:

$$(100C / V)(H_U / H_S)$$

in which *C* is the concentration, in mg per mL, of USP Hydrocortisone RS in the *Standard preparation*, *V* is the volume, in mL, of the portion of Otic Solution taken, and H_U and H_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Polymyxin B Sulfate and Trimethoprim Ophthalmic Solution

» Polymyxin B Sulfate and Trimethoprim Ophthalmic Solution is a sterile, isotonic, aqueous solution of Polymyxin B Sulfate and Trimethoprim Sulfate or of Polymyxin B Sulfate and Trimethoprim that has been solubilized with Sulfuric Acid. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of polymyxin B and the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of trimethoprim ($C_{14}H_{18}N_4O_3$). It contains one or more preservatives.

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.

Labeling—Label it to indicate that it is to be stored at 15° to 25°, protected from light.

USP Reference standards (11)—

USP Polymyxin B Sulfate RS

USP Trimethoprim RS

Identification—

A: It meets the requirements for polymyxin B under *Thin-Layer Chromatographic Identification Test* (201BNP).

B: The retention time of the trimethoprim peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for trimethoprim*.

Sterility Tests (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH (791): between 4.0 and 6.2.

Change to read:

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Ophthalmic Solution, diluted quantitatively and stepwise with *Buffer B.6* (CN 1-May-2017), to obtain a *Test Dilution* having a concentration of polymyxin B assumed to be equal to the median dose level of the Standard.

Assay for trimethoprim—

Diluent—Prepare a mixture of 0.01 N hydrochloric acid and acetonitrile (870:130).

Mobile phase—Dissolve 1.65 g of ethanesulfonic acid in 1000 mL of a mixture of water and acetonitrile (870:130). Adjust with 10 N sodium hydroxide or 0.1 N hydrochloric acid to a pH of 3.5. Pass this solution through a filter having a 0.5- μ m or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Trimethoprim RS in *Diluent* to obtain a solution having a known concentration of about 0.04 mg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 1 mg of trimethoprim, to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm \times 30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5, when calculated at 10% height of the peak; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of trimethoprim ($C_{14}H_{18}N_4O_3$) in each mL of the Ophthalmic Solution taken by the formula:

$$25(C/V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Trimethoprim RS in the *Standard preparation*; *V* is the volume, in mL, of Ophthalmic Solution taken to prepare the *Assay preparation*; and r_U and r_S are the trimethoprim peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Polyvinyl Alcohol



(C_2H_4O)_n

Ethanol, homopolymer;

Vinyl alcohol polymer [9002-89-5].

DEFINITION

Polyvinyl Alcohol is a water-soluble synthetic resin, represented by the formula (C_2H_4O)_n, in which the average value of *n* lies between 500 and 5000. It is prepared by 85%–89% hydrolysis of polyvinyl acetate. The apparent viscosity, in mPa · s, at 20°, of a 4% (w/w) aqueous solution is NLT 85.0% and NMT 115.0% of that stated on the label.

IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

• **B.** It meets the requirements in the test *Viscosity—Capillary Methods* (911), *Viscosity—Rotational Methods* (912), and *Viscosity—Rolling Ball Method* (913).

• **C.**

Sample solution: Dissolve 0.5 g of Polyvinyl Alcohol in 10 mL of water, with heat if necessary, and let the solution cool to room temperature.

Analysis 1: Transfer 5 mL of the *Sample solution* to a test tube, add 1 drop of iodine TS, mix, and allow to stand.

Acceptance criteria 1: A dark red to blue color is produced.

Analysis 2: Transfer 2 mL of the remaining *Sample solution* to a test tube, add 10 mL of Alcohol, and mix.

Acceptance criteria 2: A white, turbid, or flocculent precipitate is formed.

IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 1.0%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 µg/g • (Official 1-Jan-2018)

WATER-INSOLUBLE SUBSTANCES

Analysis: Wash the tared 100-mesh screen used in the test for Viscosity immediately after with two 25-mL portions of water, and dry at 110° for 1 h.

Acceptance criteria: NMT 6.4 mg of water-insoluble substances are found (corresponding to NMT 0.1%).

LIMIT OF METHANOL (METHYL ALCOHOL) AND METHYL ACETATE

Standard solution: In a 100-mL screw-cap bottle, prepare a 100-mL solution containing 0.24 µL/mL of USP Methyl Alcohol RS and 0.24 µL/mL of USP Methyl Acetate RS. Add 30 µL of USP Acetone RS to the screw-cap bottle. Close the bottle tightly with the screw cap, and heat it in a water bath, stirring continuously. Remove the bottle from the water bath, and allow it to cool to room temperature.

Sample solution: Transfer a quantity of Polyvinyl Alcohol, equivalent to 2.0 g on the dried basis, to a 100-mL screw-cap bottle, and add a magnetic stirrer. Add 98 mL of water and 30 µL of USP Acetone RS. Close the bottle tightly with the screw cap, and heat it in a water bath, stirring continuously. Once the solution becomes clear, remove the bottle from the water bath, and allow it to cool to room temperature.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: GC

Detector: Flame ionization

Column: 3.2-mm × 2-m glass, phase S3

Temperatures

Injection port: 160°

Column: 160°

Detector: 160°

Carrier gas: Nitrogen

Flow rate: 30 mL/min

Injection volume: 0.4 µL

System suitability

Sample: Standard solution

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentages of methanol and methyl acetate in the portion of Polyvinyl Alcohol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/W) \times V \times D \times F \times 100$$

R_U = peak response ratio of the methanol or methyl acetate peak relative to the acetone peak from the *Sample solution*

R_S = peak response ratio of the methanol or methyl acetate peak relative to the acetone peak from the *Standard solution*

C_S = concentration of methanol (methyl alcohol) or methyl acetate in the *Standard solution* (µL/mL)

W = weight of Polyvinyl Alcohol on the dried basis taken to prepare the *Sample solution* (g)

V = volume of the *Sample solution*, 100 mL

D = density of methanol (methyl alcohol) or methyl acetate, 0.79 and 0.93 g/mL, respectively

F = conversion factor, 10^{-3} mL/µL

Acceptance criteria

Methanol (methyl alcohol): NMT 1.0%

Methyl acetate: NMT 1.0%

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry at 110° to constant weight.

Acceptance criteria: NMT 5.0%

• VISCOSITY—CAPILLARY METHODS (911), VISCOSITY—ROTATIONAL METHODS (912), or VISCOSITY—ROLLING BALL METHOD (913)

Sample: After performing *Loss on Drying*, weigh undried Polyvinyl Alcohol, equivalent to 6.00 g on the dried basis.

Analysis: Over a period of seconds, transfer the *Sample* with continuous slow stirring to 140 mL of water contained in a suitable tared flask. When the specimen is well wetted, increase the rate of stirring, avoiding mixing in excess air. Heat the mixture to 90°, and maintain the temperature at 90° for about 5 min. Discontinue heating, and continue stirring for 1 h. Add water to make the mixture weigh 150 g. Resume stirring to obtain a homogeneous solution. Filter the solution through a tared 100-mesh screen into a 250-mL conical flask, cool the filtrate to 15°, mix, and proceed as directed in the chapter. Determine its viscosity at $20 \pm 0.1^\circ$, using an appropriate viscometer.

Acceptance criteria: 85.0%–115.0% of the labeled value.

• PH (791)

Sample solution: 40 mg/mL

Acceptance criteria: 5.0–8.0

• DEGREE OF HYDROLYSIS

Sample: 1 g of Polyvinyl Alcohol, previously dried at 110° to constant weight

Analysis: Transfer the *Sample* to a wide-mouth, 250-mL conical flask fitted by means of a suitable glass joint to a reflux condenser. Add 35 mL of dilute methanol (3 in 5), and mix gently to ensure complete wetting of the solid. Add 3 drops of phenolphthalein TS, and add 0.2 N hydrochloric acid or 0.2 N sodium hydroxide if necessary, to neutralize. Add 25.0 mL of 0.2 N sodium hydroxide VS, and reflux gently on a hot plate for 1 h. Wash the condenser with 10 mL of water, collecting the washings in the flask, cool, and titrate with 0.2 N hydrochloric acid VS. Concomitantly perform a blank determination in the same manner, using the same quantity of 0.2 N sodium hydroxide VS.

Calculation of saponification value: Calculate the saponification value:

$$\text{Result} = [(V_B - V_S) \times N \times M_r] / W$$

V_B = volume of 0.2 N hydrochloric acid VS consumed in the titration of the blank (mL)

V_S = volume of 0.2 N hydrochloric acid VS consumed in the titration of the *Sample solution* (mL)

N = actual normality of hydrochloric acid VS

M_r = molecular weight of potassium hydroxide, 56.11

W = weight of the portion of Polyvinyl Alcohol taken (g)

Calculation of degree of hydrolysis: Calculate the degree of hydrolysis, expressed as a percentage of hydrolysis of polyvinyl acetate:

$$\text{Result} = 100 - [7.84 \times S / (100 - 0.075 \times S)]$$

S = saponification value of the Polyvinyl Alcohol
Acceptance criteria: 85%–89%

• ACID VALUE

Sample: 10.0 g

Analysis: Add 200 mL of water to a borosilicate round-bottom flask attached to a reflux condenser. Heat the water on a water bath with constant stirring. Add the Sample to the water, and continue heating for 30 min with continuous stirring. Remove the flask from the water bath, and continue stirring until room temperature is reached. Quantitatively transfer this solution to a 250-mL volumetric flask, dilute with water to volume, and mix. Add 0.5 mL of phenolphthalein TS to 50 mL of this solution, and titrate with 0.05 N potassium hydroxide VS until the pink color persists for 15 s. Calculate the acid value:

$$\text{Result} = D \times M_r \times [(N \times V)/W]$$

D = dilution factor

M_r = molecular weight of potassium hydroxide, 56.11

N = normality of potassium hydroxide VS used

V = volume of 0.05 N potassium hydroxide used (mL)

W = weight of the Sample (g)

Acceptance criteria: NMT 3.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature in a dry place.
- **LABELING:** Label it to indicate the viscosity, giving the viscosity measurement parameters, the concentration of the solution, and the type of equipment used.
- **USP REFERENCE STANDARDS (11)**
 - USP Acetone RS
 - USP Methyl Acetate RS
 - USP Methyl Alcohol RS
 - USP Polyvinyl Alcohol RS

Sulfurated Potash

Thiosulfuric acid, dipotassium salt, mixture with potassium sulfide (K_2S_x).

Dipotassium thiosulfate mixture with potassium sulfide (K_2S_x) [39365-88-3].

» Sulfurated Potash is a mixture composed chiefly of potassium polysulfides and potassium thiosulfate. It contains not less than 12.8 percent of sulfur (S) in combination as sulfide.

Packaging and storage—Preserve in tight containers. Containers from which it is to be taken for immediate use in compounding prescriptions contain not more than 120 g.

Identification—

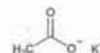
A: To a 1 in 10 solution add an excess of 6 N acetic acid: hydrogen sulfide is evolved, and sulfur is precipitated.

B: Filter the mixture from Identification test A, and add to the filtrate an excess of sodium bitartrate TS: an abundant, white, crystalline precipitate is formed within 15 minutes.

Assay for sulfides—Transfer 10 to 15 pieces of Sulfurated Potash to a mortar, and reduce to a fine powder. Transfer about 1 g of the powder, accurately weighed, to a 250-mL beaker, and dissolve in 50 mL of water. Filter, if necessary, and wash or dilute with water to 75 mL. Add, with constant stirring, 50 mL of cupric sulfate solution (1 in 20), and allow the mixture to stand, with occasional stirring, for 10 minutes. Filter through a retentive filter, and wash the precipitate with 200 mL of 0.25 N hydrochloric acid, taking care to avoid breaking up the cake. (If the filtrate is not blue in

color, discard the assay specimen, and start over, using a larger volume of cupric sulfate solution.) Ignite the precipitate in a tared dish at 1000° for 1 hour, cool in a desiccator, and weigh: the weight of the cupric oxide so obtained, multiplied by 0.4030, represents the weight of S in the specimen under assay.

Potassium Acetate



$\text{C}_2\text{H}_3\text{KO}_2$

98.14

Acetic acid, potassium salt;
Potassium acetate [127-08-2].

DEFINITION

Potassium Acetate contains NLT 99.0% and NMT 100.5% of $\text{C}_2\text{H}_3\text{KO}_2$, calculated on the dried basis.

IDENTIFICATION

• A. IDENTIFICATION TESTS—GENERAL, Potassium (191)

Sample solution: 100 mg/mL

Acceptance criteria: Meets the requirements

• B. IDENTIFICATION TESTS—GENERAL, Acetate (191)

Sample solution: 100 mg/mL

Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Sample: 200 mg of Potassium Acetate, previously dried

Analysis: Dissolve the Sample in 25 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 9.814 mg of potassium acetate ($\text{C}_2\text{H}_3\text{KO}_2$).

Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES

Delete the following:

• HEAVY METALS, Method I (231)

Test preparation: Dissolve 1 g of Potassium Acetate in 10 mL of water, add 3.0 mL of glacial acetic acid, and dilute with water to 25 mL. Adjust with glacial acetic acid to a pH of 3.8–4.0, measured with a pH meter.

Monitor preparation: Prepare as directed for the Test preparation, 2.0 mL of Standard Lead Solution being added.

Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

• LIMIT OF SODIUM

Solution A: 100 mg/mL of potassium chloride in water
Standard stock solution: Transfer 127.1 mg of sodium chloride, previously dried at 105° for 2 h into a 500-mL volumetric flask, add water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard solutions: Transfer 2.0, 5.0, and 10.0 mL of the Standard stock solution into separate 100-mL volumetric flasks, add 10.0 mL of Solution A to each flask, and dilute with water to volume. These Standard solutions contain 0.2, 0.5, and 1.0 µg/mL of sodium, respectively. [NOTE—Concentrations of sodium in the Standard solutions may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Sample solution: Transfer about 0.2 g of Potassium Acetate into a 100-mL volumetric flask containing about

50 mL of water, and swirl to dissolve. Add 10.0 mL of *Solution A*, dilute with water to volume, and mix.

[NOTE—The concentration of Potassium Acetate in the *Sample solution* may be modified by using a different quantity or by further dilution to bring the absorption response within the range of responses from the *Standard solutions*.]

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Sodium emission line at 589 nm

Lamp: Sodium hollow-cathode

Flame: Oxidizing air-acetylene

Blank solution: Transfer 10.0 mL of *Solution A* into a 100-mL volumetric flask, dilute with water to volume, and mix.

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank solution*

Determine the absorbances of the *Standard solutions* and the *Sample solution*, using the *Blank solution* to zero the instrument. Plot the absorbances of the *Standard solutions* versus the concentration, in $\mu\text{g/mL}$, of sodium, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the concentration, C , in $\mu\text{g/mL}$, of sodium in the *Sample solution*.

Calculate the percentage of sodium in the portion of Potassium Acetate taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

C = concentration of the *Sample solution*, determined from the graph ($\mu\text{g/mL}$)

W = weight of Potassium Acetate taken to prepare the *Sample solution* (g)

V = final volume of the *Sample solution*, taking into account any dilution necessary (mL)

F = conversion factor (1,000,000 $\mu\text{g/g}$)

Acceptance criteria: NMT 0.03%

SPECIFIC TESTS

• pH (791)

Sample solution: 50 mg/mL

Acceptance criteria: 7.5–8.5

• LOSS ON DRYING (731):

Dry a sample at 150° for 2 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE:

Preserve in tight containers.

Potassium Acetate Injection

» Potassium Acetate Injection is a sterile solution of Potassium Acetate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $\text{C}_2\text{H}_3\text{KO}_2$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.

Labeling—The label states the potassium acetate content in terms of weight and of milliequivalents in a given volume. Label the Injection to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration. The label states also the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before

use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

USP Reference standards (11)—

USP Endotoxin RS

Identification—It responds to the tests for *Potassium* (191) and for *Acetate* (191).

Bacterial Endotoxins Test (85)—It contains not more than 8.80 USP Endotoxin Units per mEq.

pH (791): between 5.5 and 8.0, when diluted with water to 1.0% of potassium acetate.

Particulate Matter in Injections (788): meets the requirements under small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Potassium stock solution—Dissolve 190.7 mg of potassium chloride, previously dried at 105° for 2 hours, in water. Transfer to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 100.0 mL of this solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 10 μg of potassium (equivalent to 19.07 μg of potassium chloride) per mL.

Standard preparations—To separate 100-mL volumetric flasks transfer 10.0, 15.0, and 20.0 mL, respectively, of *Potassium stock solution*. To each flask add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix. The *Standard preparations* contain, respectively, 1.0, 1.5, and 2.0 μg of potassium per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 2 g of potassium acetate, to a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the solution to a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the potassium emission line of 766.5 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a potassium hollow-cathode lamp and an air-acetylene flame, using water as the blank. Plot the absorbance of the *Standard preparation* versus concentration, in μg per mL, of potassium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, in μg per mL, of potassium in the *Assay preparation*. Calculate the quantity, in mg, of $\text{C}_2\text{H}_3\text{KO}_2$ in the portion of Injection taken by the formula:

$$500C(2.510)$$

in which C is the concentration, in μg per mL, of potassium in the *Assay preparation*, and 2.510 is the ratio of the molecular weight of potassium acetate to the atomic weight of potassium.

Potassium Bicarbonate

KHCO_3

100.12

Carbonic acid, monopotassium salt;
Monopotassium carbonate [298-14-6].

DEFINITION

Potassium Bicarbonate contains NLT 99.5% and NMT 101.5% of KHCO_3 , calculated on the dried basis.

IDENTIFICATION• **A. IDENTIFICATION TESTS—GENERAL, Potassium (191)**

Sample solution: 100 mg/mL

Acceptance criteria: Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL, Bicarbonate (191)**

Sample solution: 100 mg/mL

Acceptance criteria: Meets the requirements

ASSAY• **PROCEDURE**

Sample: 4 g

Analysis: Dissolve the Sample in 100 mL of water, add methyl red TS, and titrate with 1 N hydrochloric acid VS. Add the acid slowly, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration until the pink color no longer fades after boiling. Each mL of 1 N hydrochloric acid is equivalent to 100.1 mg of KHCO_3 .

Acceptance criteria: 99.5%–101.5% on the dried basis

IMPURITIES**Delete the following:**• **HEAVY METALS, Method I (231)**

Sample solution: To 2 g add 5 mL of water and 8 mL of 3 N hydrochloric acid, heat to boiling, and maintain that temperature for 1 min. Add 1 drop of phenolphthalein TS and sufficient 6 N ammonium hydroxide, dropwise, to give the solution a faint pink color. Cool, add 2 mL of 1 N acetic acid, and then dilute with water to 25 mL.

Acceptance criteria: NMT 10 ppm (Official 1-Jan-2018)

SPECIFIC TESTS• **LOSS ON DRYING (731):** Dry a sample over silica gel for 4 h: it loses NMT 0.3% of its weight.• **NORMAL CARBONATE**

Sample: Grind 3.0 g of Potassium Bicarbonate with 25 mL of alcohol and 5 mL of water in a porcelain mortar.

Barium chloride solution: 12.216 mg/mL of barium chloride in alcohol and water (7:3), prepared by dissolving the barium chloride in water, then diluting with alcohol to final volume

Analysis: Add 3 drops of phenolphthalein TS to the Sample, and titrate slowly with Barium chloride solution until the suspension becomes colorless. Continue the grinding for 2 min, and if the color turns pink, continue the titration with Barium chloride solution to a colorless endpoint. Repeat the grinding for 2 min and the addition of Barium chloride solution, if necessary, until the suspension is colorless after 2 min of grinding. Each mL of the barium chloride solution is equivalent to 6.911 mg of K_2CO_3 .

Acceptance criteria: NMT 2.5%

ADDITIONAL REQUIREMENTS• **PACKAGING AND STORAGE:** Preserve in well-closed containers.**Potassium Bicarbonate Effervescent Tablets for Oral Solution**

» Potassium Bicarbonate Effervescent Tablets for Oral Solution contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of K.

Packaging and storage—Preserve in tight containers, protected from excessive heat.

Labeling—The label states the potassium content in terms of weight and in terms of milliequivalents. Where the Tablets are packaged in individual pouches, the label instructs the user not to open until the time of use.

Identification—One Tablet dissolves in 100 mL of water with effervescence. The collected gas responds to the test for Bicarbonate (191), and the resulting solution responds to the test for Potassium (191).

Uniformity of dosage units (905): meet the requirements.

Assay—

Standard stock solution and Standard solutions—Prepare as directed in the Assay under Potassium Chloride Oral Solution.

Assay preparation—Transfer 10 Tablets to a 2000-mL volumetric flask, dissolve in 200 mL of water, swirl until effervescence ceases, dilute with water to volume, and mix. Filter, and quantitatively dilute an accurately measured volume of the filtrate with water to obtain a solution containing 30 µg of potassium per mL. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Procedure—Proceed as directed for Instrumental conditions and Analysis in the Assay under Potassium Chloride Oral Solution, except use Assay preparation instead of Sample solution. Calculate the quantity, in mg, of K in each Tablet taken by the formula:

$$L(C/D)$$

in which *L* is the labeled quantity, in mg, of potassium in each Tablet, *C* is the concentration, in µg per mL, of potassium in the Assay preparation, and *D* is the concentration, in µg per mL, of potassium in the Assay preparation on the basis of the labeled quantity in each Tablet and the extent of dilution.

Potassium Bicarbonate and Potassium Chloride for Effervescent Oral Solution

» Potassium Bicarbonate and Potassium Chloride for Effervescent Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of K and Cl.

Packaging and storage—Preserve in tight containers, protected from excessive heat.

Labeling—The label states the potassium and chloride contents in terms of weight and in terms of milliequivalents. Where packaged in individual pouches, the label instructs the user not to open until the time of use.

Identification—A 3-g portion dissolves in 100 mL of water with effervescence. The collected gas so obtained responds to the test for Bicarbonate (191), and the resulting solution responds to the tests for Potassium (191) and for Chloride (191).

Minimum fill (755)—

FOR SOLID PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Uniformity of dosage units (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Assay for potassium—

Standard stock solution and Standard solutions—Prepare as directed in the Assay under Potassium Chloride Oral Solution.

Assay preparation—Weigh and mix the contents of not less than 20 containers of Potassium Bicarbonate and Potassium Chloride for Effervescent Oral Solution. Transfer an accurately weighed portion of the powder, equivalent to about 6 g of potassium, to a 1000-mL volumetric flask, dissolve in about 200 mL of water, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a second 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Procedure—Proceed as directed for *Instrumental conditions* and *Analysis* in the *Assay* under *Potassium Chloride Oral Solution*, except use *Assay preparation* instead of *Sample solution*. Calculate the quantity, in mg, of K in the portion of Potassium Bicarbonate and Potassium Chloride for Effervescent Oral Solution taken by the formula:

$$400C$$

in which *C* is the concentration, in μg per mL, of potassium in the *Assay preparation*.

Assay for chloride—Weigh and mix the contents of not less than 20 containers of Potassium Bicarbonate and Potassium Chloride for Effervescent Oral Solution. Transfer a portion of the powder, equivalent to about 900 mg of chloride, to a 2000-mL volumetric flask. Add about 200 mL of water, swirl until effervescence ceases, dilute with water to volume, and mix. Transfer 25.0 mL of this solution to a 250-mL conical flask, add 50.0 mL of 0.1 N silver nitrate VS and 15 mL of nitric acid, and boil, with constant swirling, until the solution is colorless. Cool to room temperature, add water to make about 150 mL, then add 5 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS to a permanent faint brown endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl.

Potassium Bicarbonate and Potassium Chloride Effervescent Tablets for Oral Solution

» Potassium Bicarbonate and Potassium Chloride Effervescent Tablets for Oral Solution contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of K and Cl.

Packaging and storage—Preserve in tight containers, protected from excessive heat.

Labeling—The label states the potassium and chloride contents in terms of weight and in terms of milliequivalents. Where the Tablets are packaged in individual pouches, the label instructs the user not to open until the time of use.

Identification—One Tablet dissolves in 100 mL of water with effervescence. The collected gas responds to the test for Bicarbonate (191), and the resulting solution responds to the tests for Potassium (191) and for Chloride (191).

Uniformity of dosage units (905): meet the requirements.

Assay for potassium—

Standard stock solution and Standard solutions—Prepare as directed in the *Assay* under *Potassium Chloride Oral Solution*.

Assay preparation—Transfer 10 Tablets to a 2000-mL volumetric flask, dissolve in 200 mL of water, swirl until effervescence ceases, dilute with water to volume, and mix. Filter, and quantitatively dilute an accurately measured volume of

the filtrate with water to obtain a solution containing 30 μg of potassium per mL. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Procedure—Proceed as directed for *Instrumental conditions* and *Analysis* in the *Assay* under *Potassium Chloride Oral Solution*, except use *Assay preparation* instead of *Sample solution*. Calculate the quantity, in mg, of K in each Tablet taken by the formula:

$$L(C/D)$$

in which *L* is the labeled quantity, in mg, of potassium in each Tablet, *C* is the concentration, in μg per mL, of potassium in the *Assay preparation*, and *D* is the concentration, in μg per mL, of potassium in the *Assay preparation* on the basis of the labeled quantity in each Tablet and the extent of dilution.

Assay for chloride—Transfer a number of Tablets, equivalent to about 900 mg of chloride, to a 2000-mL volumetric flask. Add about 200 mL of water, swirl until effervescence ceases, dilute with water to volume, and mix. Transfer 25.0 mL of this solution to a 250-mL conical flask, add 50.0 mL of 0.1 N silver nitrate VS and 15 mL of nitric acid, and boil, with constant swirling, until the supernatant is colorless. Cool to room temperature, add sufficient water to make a volume of about 150 mL, add 5 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS to a permanent faint brown endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Calculate the quantity, in mg, of chloride (Cl) in each Tablet by dividing the total amount of chloride in the Tablets taken by the number of Tablets taken.

Potassium and Sodium Bicarbonates and Citric Acid Effervescent Tablets for Oral Solution

DEFINITION

Potassium and Sodium Bicarbonates and Citric Acid Effervescent Tablets for Oral Solution contain NLT 90.0% and NMT 110.0% of the labeled amounts of potassium bicarbonate (KHCO_3), sodium bicarbonate (NaHCO_3), and anhydrous citric acid ($\text{C}_6\text{H}_8\text{O}_7$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL**, Bicarbonate (191), Potassium (191), Sodium (191), and Citrate (191)

Sample: One Tablet for Oral Solution

Analysis 1: Dissolve the sample in 100 mL of water, and collect the gas that evolves.

Acceptance criteria 1: The *Sample* effervesces when dissolved.

Analysis 2: Proceed as directed in *Bicarbonate* (191) on the gas collected from *Analysis 1*.

Acceptance criteria 2: Meets the requirements

Analysis 3: Proceed as directed in *Potassium* (191) and *Sodium* (191) on the resulting solution from *Analysis 1*.

Acceptance criteria 3: Meets the requirements

Analysis 4: Proceed as directed in *Citrate* (191) on 3–5 drops of the resulting solution from *Analysis 1* and 20 mL of the mixture of pyridine and acetic anhydride.

Acceptance criteria 4: Meets the requirements

ASSAY

- **POTASSIUM BICARBONATE AND SODIUM BICARBONATE**

Potassium chloride stock solution: 7.5 mg/mL of potassium chloride, previously dried at 125° for 30 min, in water

Sodium chloride stock solution: 7 mg/mL of sodium chloride, previously dried at 125° for 30 min, in water
Diluent: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Before dilution to final volume, add a suitable nonionic surfactant.

Standard stock solution: 0.75 mg/mL of potassium chloride and 0.7 mg/mL of sodium chloride in water from *Potassium chloride stock solution* and *Sodium chloride stock solution*, respectively

Standard solution: 0.0375 mg/mL of potassium chloride and 0.035 mg/mL of sodium chloride in *Diluent* from the *Standard stock solution*

Sample stock solution A: Nominally 3 mg/mL of potassium bicarbonate prepared as follows. Finely powder NLT 20 Tablets for Oral Solution. Tablets for Oral Solution and powder are hygroscopic. After removal from the container, grind the Tablets for Oral Solution promptly in an atmosphere of low relative humidity, and weigh the powder promptly. Transfer a portion of the powder, equivalent to about 3000 mg of potassium bicarbonate, to a 1000-mL volumetric flask. Dissolve in 500 mL of water, swirl until effervescence ceases, and dilute with water to volume.

Sample stock solution B: Nominally 1 mg/mL of potassium bicarbonate, based on the labeled quantity, from *Sample stock solution A* in water

Sample stock solution C: Nominally 1 mg/mL of sodium bicarbonate, based on the labeled quantity, from *Sample stock solution A* in water

Sample solution A: Nominally 0.05 mg/mL of potassium bicarbonate from *Sample stock solution B* in *Diluent*

Sample solution B: Nominally 0.05 mg/mL of sodium bicarbonate from *Sample stock solution C* in *Diluent*

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: The maximum at about 766 nm

Sodium: The maximum at about 589 nm

Blank: *Diluent*

Analysis

Samples: *Standard solution*, *Sample solution A*, *Sample solution B*, and *Blank*

Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution*, *Sample solution A*, and *Sample solution B*.

Calculate the percentage of the labeled amount of potassium bicarbonate (KHCO₃) in the portion of Tablets for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = photometer reading of potassium from *Sample solution A*

r_S = photometer reading of potassium from the *Standard solution*

C_S = concentration of potassium chloride in the *Standard solution* (mg/mL)

C_U = nominal concentration of potassium bicarbonate in *Sample solution A* (mg/mL)

M_{r1} = molecular weight of potassium bicarbonate, 100.12

M_{r2} = molecular weight of potassium chloride, 74.55
 Calculate the percentage of the labeled amount of sodium bicarbonate (NaHCO₃) in the portion of Tablets for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = photometer reading of sodium from *Sample solution B*

r_S = photometer reading of sodium from the *Standard solution*

C_S = concentration of sodium chloride in the *Standard solution* (mg/mL)

C_U = nominal concentration of sodium bicarbonate in *Sample solution B* (mg/mL)

M_{r1} = molecular weight of sodium bicarbonate, 84.01

M_{r2} = molecular weight of sodium chloride, 58.44

Acceptance criteria: 90.0%–110.0% of the labeled amounts of potassium bicarbonate (KHCO₃) and sodium bicarbonate (NaHCO₃)

• ANHYDROUS CITRIC ACID

Mobile phase, Standard preparation 1, and Chromatographic system: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345).

Sample solution: Nominally 20 µg/mL of citrate prepared as follows. Transfer the equivalent of 40 mg of anhydrous citric acid from *Sample stock solution A* in the *Assay for Potassium Bicarbonate and Sodium Bicarbonate* into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345), *Assay Preparation for Citric Acid/Citrate Assay*.

Analysis:

Samples: *Standard preparation 1* and *Sample solution*
 Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345), *Procedure*.

Calculate the percentage of the labeled amount of anhydrous citric acid (C₆H₈O₇) in the portion of Tablets for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of citrate from the *Sample solution*

r_S = peak response of citrate from *Standard preparation 1*

C_S = concentration of citrate in *Standard preparation 1* (µg/mL)

C_U = nominal concentration of citrate in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of anhydrous citric acid, 192.12

M_{r2} = molecular weight of citrate, 189.10

Acceptance criteria: 90.0%–110.0%

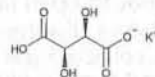
SPECIFIC TESTS

- **ACID-NEUTRALIZING CAPACITY (301):** The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label Tablets for Oral Solution to state the sodium content. The label states also that the tablets are to be dissolved in water before being taken.
- **USP REFERENCE STANDARD (11)**
 USP Citric Acid RS

Potassium Bitartrate



C₄H₅KO₆ 188.18
 Butanedioic acid 2,3-dihydroxy-, [R-(R*,R*)]-, monopotassium salt;
 Potassium hydrogen tartrate [868-14-4].

DEFINITION

Potassium Bitartrate, dried at 105° for 3 h, contains NLT 99.0% and NMT 101.0% of C₄H₅KO₆.

IDENTIFICATION

- **A.** Ignite it: it leaves a residue that imparts a reddish purple color to a nonluminous flame.
- **B.** A saturated solution of it yields a yellowish orange precipitate with sodium cobaltinitrite TS.
- **C. IDENTIFICATION TESTS—GENERAL, Tartrate (191):** Meets the requirements

ASSAY**• PROCEDURE**

Sample: Dry 6 g of Potassium Bitartrate at 105° for 3 h. Allow to cool, and weigh.

Analysis: Dissolve the *Sample* in 100 mL of boiling water, add a few drops of phenolphthalein TS, and titrate with 1 N sodium hydroxide VS to a pink endpoint. Perform a blank determination (see *Titrimetry* (541)). Each mL of 1 N sodium hydroxide is equivalent to 188.2 mg of $C_4H_5KO_6$.

Acceptance criteria: 99.0%–101.0% on the previously dried basis

IMPURITIES**• INSOLUBLE MATTER**

Sample: 500 mg

Analysis: Mix the *Sample* with 3 mL of 6 N ammonium hydroxide.

Acceptance criteria: No undissolved residue remains.

• LIMIT OF AMMONIA

Sodium hypochlorite solution: Use a commercially available solution that contains 4.0%–6.0% of sodium hypochlorite.

Oxidizing solution: [NOTE—Prepare on the day of use.] Alkaline sodium citrate TS and *Sodium hypochlorite solution* (4:1)

Diluted sodium nitroferricyanide solution: Sodium nitroferricyanide TS and water (1:10)

Standard stock solution: Dry 300 mg of ammonium chloride over silica gel for 4 h, and use it to prepare a 0.3 mg/mL solution in water. This solution contains 100 µg/mL of ammonia.

Standard solution: 0.25 µg/mL of ammonia in water, from *Standard stock solution*

Sample solution: 2.5 mg/mL of Potassium Bitartrate in water. Heat gently to facilitate the dissolution.

Analysis

[NOTE—Carefully follow the order of addition stated below.]

Separately transfer 6.0 mL each of the *Standard solution* and the *Sample solution* to two color-comparison tubes. To each tube add 0.4 mL of phenol TS, 0.4 mL of *Diluted sodium nitroferricyanide solution*, and 1.0 mL of *Oxidizing solution*. Dilute with water to 10 mL, mix, and allow to stand for 1 h.

Acceptance criteria: The color of the *Sample solution* is not darker than the color of the *Standard solution* (NMT 0.01%).

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm • (Official 1-Jan-2018)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Bromide

KBr 119.00

Potassium bromide.

Potassium bromide [7758-02-3].

» Potassium Bromide contains not less than 98.0 percent and not more than 100.5 percent of KBr, calculated on the dried basis. It contains no added substances.

Packaging and storage—Preserve in well-closed containers, and store at room temperature.

Appearance of solution: clear and colorless.

Test solution—Dissolve 10.0 g in carbon dioxide-free water, and dilute with the same solvent to 100 mL.

Identification—

A: A solution containing 4.5 mg of potassium bromide responds to the test for *Bromide* (191).

B: Responds to the test for *Potassium* (191).

Acidity or alkalinity—To 10 mL of the solution prepared for the test for *Appearance of solution*, add 0.1 mL of bromothymol blue TS: not more than 0.5 mL of 0.01 N hydrochloric acid or 0.01 N sodium hydroxide is required to change the color of this solution.

Loss on drying (731)—Dry it at 100° to 105° for 3 hours: it loses not more than 1.0% of its weight.

Bromates—

Starch—mercuric iodide solution—Triturate 1.0 g of soluble starch with 5 mL of water and pour the mixture into 100 mL of boiling water, containing 10 mg of mercuric iodide.

Procedure—To 10 mL of the solution prepared for the test for *Appearance of solution* add 1 mL of *Starch—mercuric iodide solution*, 0.1 mL of a 100 g per L solution of potassium iodide, and 0.25 mL of 0.5 M sulfuric acid. Allow to stand protected from light for 5 minutes: no blue or violet color develops.

Limit of chlorine: not more than 0.6%.

Nitric acid solution and Ferric ammonium sulfate solution—Proceed as directed in the Assay.

Procedure—Dissolve 1.000 g of Potassium Bromide in 20 mL of *Nitric acid solution* in a conical flask, add and mix 5 mL of 30 percent hydrogen peroxide, and heat in a water bath until the solution is colorless. Rinse the sides of the flask with a small quantity of water, and heat in a water bath for 15 minutes. Allow to cool, dilute with water to 50 mL, and add 5.0 mL of silver nitrate VS and 1 mL of dibutyl phthalate. Mix, and back titrate the excess silver nitrate with ammonium thiocyanate VS (see *Titrimetry* (541)), using 5 mL of *Ferric ammonium sulfate solution* as the indicator. Perform a blank titration. Not more than 1.7 mL of silver nitrate VS is used.

Iodides—To 5 mL of the solution prepared for the test for *Appearance of solution* add 0.15 mL of a 10.5 g per 100 mL ferric chloride solution, and 2 mL of dichloromethane. Shake, and allow to separate. The lower layer is colorless.

Sulfates (221)—A 2.0-g portion shows no more sulfate than corresponds to 0.2 mL of 0.020 N sulfuric acid (0.01%).

Limit of iron: not more than 20 ppm.

Citric acid solution—Prepare a 200-mg citric acid per mL solution.

Iron standard solution—Transfer 0.863 g of ferric ammonium sulfate to a 500-mL volumetric flask, and dissolve in 25 mL of dilute sulfuric acid. Dilute with water to volume. Transfer 1.0 mL of the resulting solution to a 10-mL volumetric flask, and dilute with water to volume. Transfer 2.5 mL of this resulting solution to a 50-mL volumetric flask, and dilute with water to volume. [NOTE—Prepare immediately before use.]

Test solution—Transfer 5 mL of the solution prepared for the test for *Appearance of solution* to a 10-mL volumetric flask, and dilute with water to volume.

Procedure—To 10 mL each of the *Iron standard solution* and the *Test solution* add 2.0 mL of the *Citric acid solution*

and 0.1 mL of thioglycolic acid. Make alkaline to litmus with ammonia water, and dilute with water to 20 mL. After 5 minutes, any pink color in the *Test solution* is not more intense than that in the *Iron standard solution*.

Magnesium and alkaline-earth metals—To 200 mL of water add 0.1 g of hydroxylamine hydrochloride, 10 mL of pH 10.0 ammonia–ammonium chloride buffer (prepared by dissolving 5.4 g of ammonium chloride in 20 mL of water, adding 20 mL of ammonium hydroxide and diluting to 100 mL), 1 mL of 0.1 M zinc sulfate, and about 0.2 g of eriochrome black T titration. Heat to about 40°. Titrate this solution (see *Titrimetry* (541)) with 0.01 M edetate disodium VS until the violet color changes to deep blue. To this solution add 10.0 g of Potassium Bromide dissolved in 100 mL of water. If the color changes to violet, titrate the solution with 0.01 M edetate disodium VS to a deep blue endpoint. The volume of 0.01 M edetate disodium consumed in the second titration does not exceed 5.0 mL (0.02%, calculated as Ca).

Delete the following:

• **Heavy metals, Method I (231):** not more than 10 ppm.

• (Official 1-Jan-2018)

Assay—

Nitric acid solution—Dilute 14 mL of nitric acid with water to 100 mL.

Ferric ammonium sulfate solution—Transfer 10 g of ferric ammonium sulfate to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.

Procedure—Dissolve 2.000 g of Potassium Bromide in water, and dilute with water to 100.0 mL. To 10.0 mL of the solution add 50 mL of water, 5 mL of *Nitric acid solution*, 25.0 mL of silver nitrate VS, and 2 mL of dibutyl phthalate. Mix, and back titrate the excess silver nitrate with ammonium thiocyanate VS (see *Titrimetry* (541)), using 2 mL of *Ferric ammonium sulfate solution* as the indicator, shaking vigorously towards the endpoint. Each mL of 0.1 M silver nitrate is equivalent to 11.90 mg of KBr. Calculate the percent content of Potassium Bromide, corrected for the chloride content, by the formula:

$$a - 3.357b$$

in which *a* is the percent content of KBr and KCl obtained, calculated as KBr; and *b* is the percent content of chlorides.

Potassium Bromide Compounded Oral Solution, Veterinary

DEFINITION

Potassium Bromide Compounded Oral Solution, Veterinary contains NLT 225 mg and NMT 275 mg of potassium bromide (KBr) per mL, equivalent to NLT 151 mg and NMT 185 mg of bromide (Br⁻) per mL.

Prepare Potassium Bromide Compounded Oral Solution, Veterinary as follows (see *Pharmaceutical Compounding—Non-sterile Preparations* (795)).

Potassium Bromide	25 g
Purified Water	60 mL
Corn Syrup, FCC, a sufficient quantity to make	100 mL

Dissolve the *Potassium Bromide* in *Purified Water*. Add *Corn Syrup*, FCC, to bring to final volume with mixing.

ASSAY

• PROCEDURE

TCA solution: 20% (w/v) trichloroacetic acid in water
Gold chloride solution: 5 mg/mL of gold chloride in water

Standard stock solution: Dissolve USP Sodium Bromide RS in water to obtain a solution with a nominal concentration of 20 mg/mL of bromide.

Standard solutions: Prepare four solutions of known concentrations of about 2.0, 1.0, 0.5, and 0.25 mg/mL of bromide by diluting the *Standard stock solution* with water.

Sample solution: Dilute Oral Solution, Veterinary quantitatively with water (1:99).

Blank: Water

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: Vis

Analytical wavelength: 440 nm

System suitability

Samples: *Standard solutions* and *Blank*

Suitability requirements

Correlation coefficient: NLT 0.99, linear regression of the *Standard solutions*

Analysis

Samples: *Sample solution* and *Blank*

To 750-μL aliquots of each *Sample* add 500 μL of TCA solution and 250 μL of *Gold chloride solution*. Mix on a vortex mixer, and immediately read the absorbance of each *Sample*.

Calculate the concentration, in mg/mL, of bromide (Br⁻) in the portion of Oral Solution, Veterinary taken:

$$\text{Result} = C \times D$$

C = concentration of the *Sample solution* (mg/mL) obtained from the standard curve

D = dilution factor of the *Sample solution*, 100

Acceptance criteria: 151–185 mg/mL of bromide (Br⁻)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in a tight container, and store in a refrigerator.
- **BEYOND-USE DATE:** NMT 180 days after the date on which it was compounded when stored in a refrigerator
- **LABELING:** Label it to state the *Beyond-Use Date*. Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS (11)**
USP Sodium Bromide RS

Potassium Carbonate

K₂CO₃ (anhydrous) 138.21

K₂CO₃ · 1½H₂O 165.23

Carbonic acid, dipotassium salt;
Dipotassium carbonate [584-08-7].

DEFINITION

Potassium Carbonate contains NLT 99.5% and NMT 100.5% of K₂CO₃, calculated on the dried basis.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Potassium (191):**
Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL, Carbonate (191):**
Meets the requirements

ASSAY

• PROCEDURE

Sample: Dried potassium carbonate obtained in the test for *Loss on Drying*

Analysis: Transfer the *Sample* to a flask with the aid of 150 mL of water, and add 4 drops of methyl orange TS. Titrate with 1 N hydrochloric acid VS. Each mL of 1 N hydrochloric acid is equivalent to 69.11 mg of K_2CO_3 .

Acceptance criteria: 99.5%–100.5% on the dried basis

IMPURITIES

Delete the following:

• HEAVY METALS (231)

Analysis: Dissolve 4.0 g in 10 mL of water. Add 15 mL of 3 N hydrochloric acid, and heat to boiling. Add 1 drop of phenolphthalein TS, and neutralize with 1 N sodium hydroxide until the solution is faintly pink in color. Cool, and dilute with water to 25 mL.

Acceptance criteria: NMT 5 ppm • (Official 1-Jan-2018)

SPECIFIC TESTS

- **LOSS ON DRYING (731):** Dry a sample at 180° for 4 h: it loses NMT 0.5% of its weight.

• INSOLUBLE SUBSTANCES

Sample solution: 1 g in 20 mL of water

Acceptance criteria: The solution is complete, clear, and colorless.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Potassium Chloride

KCl 74.55
Potassium chloride [7447-40-7].

DEFINITION

Potassium Chloride contains NLT 99.0% and NMT 100.5% of KCl, calculated on the dried basis.

IDENTIFICATION

• A. IDENTIFICATION TESTS—GENERAL, Potassium (191)

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

• B. IDENTIFICATION TESTS—GENERAL, Chloride (191)

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Sample: 200 mg

Analysis: Dissolve the *Sample* in 10 mL of water. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 7.455 mg of KCl.

Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES

- **ALUMINUM (206)** (where it is labeled as intended for use in hemodialysis): Proceed as directed, using 2.0 g of Potassium Chloride to prepare the *Test preparation*.

Acceptance criteria: NMT 1 ppm

• SODIUM

Sample solution: 50 mg/mL

Acceptance criteria: *Sample solution* tested on a platinum wire does not impart a pronounced yellow color to a nonluminous flame.

• IODIDE

Standard stock solution: 1.64 mg/mL of potassium iodide in water

Standard solution: Dilute 1.0 mL of *Standard stock solution* with water to 25 mL. Dilute 2.0 mL of this solution with water to 8 mL. Add 1 mL each of chloroform and diluted hydrochloric acid, then add 2 drops of a chloramine T solution (0.1 in 100), and shake gently.

Sample solution: Dissolve 2 g of Potassium Chloride in 8 mL of water. Add 1 mL each of chloroform and diluted hydrochloric acid, then add 2 drops of a chloramine T solution (0.1 in 100), and shake gently.

Acceptance criteria: The violet color of the chloroform layer is not darker than that of a concomitantly prepared *Standard solution* (NMT 0.005%).

• BROMIDE

Standard stock solution: 1.28 mg/mL of sodium bromide in water

Standard solution: Dilute 2.0 mL of *Standard stock solution* with water to 8 mL. Add 1 mL each of chloroform and diluted hydrochloric acid, then add 5 drops of a chloramine T solution (1 in 100), and shake gently.

Sample solution: Dissolve 2 g of Potassium Chloride in 8 mL of water. Add 1 mL each of chloroform and diluted hydrochloric acid, then add 5 drops of a chloramine T solution (1 in 100), and shake gently.

Acceptance criteria: The brown color of the chloroform layer is not darker than that of a concomitantly prepared *Standard solution* (NMT 0.1%).

• CALCIUM AND MAGNESIUM

Sample solution: 10 mg/mL in water

Analysis: To 20 mL of *Sample solution* add 2 mL each of 6 N ammonium hydroxide, ammonium oxalate TS, and dibasic sodium phosphate TS.

Acceptance criteria: No turbidity is produced within 5 min.

Delete the following:

• HEAVY METALS (231)

Sample solution: 2.0 g in 25 mL of water

Acceptance criteria: NMT 10 ppm • (Official 1-Jan-2018)

SPECIFIC TESTS

- **ACIDITY OR ALKALINITY:** To a solution of 5.0 g in 50 mL of carbon dioxide-free water add 3 drops of phenolphthalein TS: no pink color is produced. Then add 0.30 mL of 0.020 N sodium hydroxide: a pink color is produced.
- **LOSS ON DRYING (731):** Dry a sample at 105° for 2 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Where Potassium Chloride is intended for use in hemodialysis, it is so labeled.

Potassium Chloride Extended-Release Capsules

» Potassium Chloride Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of KCl.

Packaging and storage—Preserve in tight containers at a temperature not exceeding 30°.

Identification—A portion of the filtrate obtained as directed under *Assay* in the *Assay* responds to the tests for *Potassium (191)* and for *Chloride (191)*.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 2 hours.

Standard stock solution and Standard solutions—Prepare as directed in the Assay under *Potassium Chloride Oral Solution*.

Procedure—Filter the solution under test, and dilute quantitatively with *Dissolution Medium* to obtain a test solution containing about 60 µg of potassium chloride per mL. Add 5.0 mL of the test solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, mix, and proceed as directed for *Instrumental conditions and Analysis* in the Assay under *Potassium Chloride Oral Solution*. Calculate the quantity, in mg, of KCl dissolved by the formula:

$$(900F)(1.907C)$$

in which *F* is the extent of dilution of the solution under test, and the other terms are as defined therein.

Tolerances—Not more than 35% (*Q*) of the labeled amount of KCl is dissolved in 2 hours. The requirements are met if the quantities dissolved from the Capsules tested conform to the accompanying acceptance table instead of the table shown under *Dissolution* (711).

Acceptance Table

Stage	Number Tested	Acceptance Criteria
S ₁	6	Each unit is within the range $Q \pm 30\%$.
S ₂	6	Average of 12 units ($S_1 + S_2$) is within the range between $Q - 30\%$ and $Q + 35\%$, and no unit is outside the range $Q \pm 40\%$.
S ₃	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between $Q - 30\%$ and $Q + 35\%$, and not more than 2 units are outside the range $Q \pm 40\%$.

Uniformity of dosage units (905): meet the requirements.

Assay—

Standard stock solution and Standard solutions—Prepare as directed in the Assay under *Potassium Chloride Oral Solution*.

Assay preparation—Place not less than 20 Capsules in a suitable container with 400 mL of water, heat to boiling, and boil for 20 minutes. Allow to cool, transfer the solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. Filter, discarding the first 20 mL of the filtrate. Transfer an accurately measured volume of the subsequent filtrate, equivalent to about 60 mg of potassium chloride, to a 1000-mL volumetric flask, dilute with water to volume, and mix. (Retain a portion of the filtrate for use in the *Identification* test.) Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Procedure—Proceed as directed for *Instrumental conditions and Analysis* in the Assay under *Potassium Chloride Oral Solution*, except use *Assay preparation* instead of *Sample solution*. Calculate the quantity, in mg, of KCl in each Capsule taken by the formula:

$$(TC/D)(1.907)$$

in which *T* is the labeled quantity, in mg, of potassium chloride in each Capsule, *D* is the concentration, in µg per mL, of potassium chloride in the *Assay preparation*, based on the labeled quantity per Capsule and the extent of dilution, and the other terms are as defined therein.

Potassium Chloride for Injection Concentrate

» Potassium Chloride for Injection Concentrate is a sterile solution of Potassium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of KCl.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.

Labeling—The label states the potassium chloride content in terms of weight and of milliequivalents in a given volume. Label the Concentrate to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration. Immediately following the name, the label bears the boxed warning:

Concentrate Must be Diluted Before Use

This warning is not required when the liquid preparation is in a *Pharmacy bulk package* and the label thereon states prominently "Pharmacy Bulk Package—Not for direct infusion."

The cap of the container and the overseal of the cap must be black, and both bear the words: "Must Be Diluted" in readily legible type, in a color that stands out from its background OR the overseal may be of a clear plastic material through which the black cap is visible and the printing is readily legible.

When the nature of the container-closure system prevents compliance, the design shall follow the intent of this requirement as closely as possible, the black color being used beneath the words "Must be Diluted," which are so placed that words are readily visible as the contents of the container are being removed. Ampuls shall be identified by a black band or a series of black bands above the constriction. The label states also the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol per mL.

USP Reference standards (11)—

USP Endotoxin RS

Identification—It responds to the tests for *Potassium* (191) and for *Chloride* (191).

Bacterial Endotoxins Test (85): It contains not more than 8.80 USP Endotoxin Units per mEq.

pH (791): between 4.0 and 8.0.

Particulate Matter in Injections (788): meets the requirements under small-volume injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay—

Potassium stock solution and Standard preparations—Prepare as directed in the Assay under *Potassium Chloride Oral Solution*.

Assay preparation—Transfer an accurately measured volume of Concentrate, equivalent to about 600 mg of potassium chloride, to a 500-mL volumetric flask, dilute with water to volume, and mix. Proceed as directed for *Assay preparation* in the Assay under *Potassium Chloride Oral Solution*, beginning with "Transfer 5.0 mL of the solution to a 100-mL volumetric flask."

Procedure—Proceed as directed for *Procedure* in the Assay under *Potassium Chloride Oral Solution*. Calculate the quan-

tity, in mg, of KCl in the portion of Concentrate taken by the formula:

$$200C(1.907)$$

in which the terms are as defined therein.

Potassium Chloride Oral Solution

DEFINITION

Potassium Chloride Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of potassium chloride (KCl). It may contain alcohol.

IDENTIFICATION

• A. IDENTIFICATION TESTS—GENERAL, Potassium (191)

Sample solution: Carefully evaporate 5 mL to dryness, and ignite the residue at dull-red heat to remove all organic matter. Cool, dissolve the residue in 10 mL of water, and filter.

Acceptance criteria: Meets the requirements

• B. IDENTIFICATION TESTS—GENERAL, Chloride (191)

Sample solution: Carefully evaporate 5 mL to dryness, and ignite the residue at dull-red heat to remove all organic matter. Cool, dissolve the residue in 10 mL of water, and filter.

Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Standard stock solution: 19.07 µg/mL of potassium chloride, previously dried at 105° for 2 h, in water. This solution contains 10 µg/mL of potassium.

Standard solutions: To separate 100-mL volumetric flasks transfer 10.0, 15.0, and 20.0 mL, respectively, of *Standard stock solution*. To each flask add 2.0 mL of sodium chloride solution (200 mg/mL) and 1.0 mL of hydrochloric acid, and dilute with water to volume. The *Standard solutions* contain, respectively, 1.0, 1.5, and 2.0 µg/mL of potassium.

Sample stock solution: Transfer a volume of Oral Solution, equivalent to 600 mg of potassium chloride, to a 500-mL volumetric flask, and dilute with water to volume. Transfer 5.0 mL of the solution to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (200 mg/mL) and 1.0 mL of hydrochloric acid, and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Potassium emission line at 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air-acetylene

Blank: Water

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*. Plot the absorbance of the *Standard solutions* versus the concentration of potassium, in µg/mL, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration of potassium in the *Sample solution* (µg/mL).

Calculate the percentage of the labeled amount of potassium chloride (KCl) in the portion of Oral Solution taken:

$$\text{Result} = (C/C_u) \times (M_r/A_r) \times 100$$

C = concentration of potassium in the *Sample solution* as determined in this test (µg/mL)

C_u = nominal concentration of potassium chloride in the *Sample solution* (µg/mL)

M_r = molecular weight of potassium chloride, 74.55

A_r = atomic weight of potassium, 39.10

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

- **ALCOHOL DETERMINATION, Method II (611)** (if present): NLT 90.0% and NMT 115.0% of the labeled amount, the labeled amount being NMT 7.5% of C_2H_5OH , acetone being used as the internal standard

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Chloride for Oral Solution

» Potassium Chloride for Oral Solution is a dry mixture of Potassium Chloride and one or more suitable colors, diluents, and flavors. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of KCl.

Packaging and storage—Preserve in tight containers.

Labeling—The label states the Potassium Chloride (KCl) content in terms of weight and in terms of milliequivalents.

Identification—Ignite about 200 mg at a temperature not above 600°, in order to remove all organic matter, cool, dissolve the residue in 10 mL of water, and filter: the filtrate responds to the tests for *Potassium* (191) and for *Chloride* (191).

Minimum fill (755)—

FOR SOLID PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Uniformity of dosage units (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Assay—

Standard stock solution and *Standard solutions*—Prepare as directed in the Assay under *Potassium Chloride Oral Solution*.

Assay preparation 1 (where it is packaged in unit-dose containers)—Weigh and mix the contents of not less than 20 containers of Potassium Chloride for Oral Solution. Transfer an accurately weighed portion of the powder, equivalent to about 1.5 g of potassium chloride, to a 500-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. Transfer 5.0 mL of the solution to a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Assay preparation 2 (where it is packaged in multiple-unit containers)—Transfer an accurately weighed portion of Potassium Chloride for Oral Solution, equivalent to about 1.5 g of potassium chloride, to a 500-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. Proceed as directed for *Assay preparation 1*, beginning with "Transfer 5.0 mL of the solution."

Procedure—Proceed as directed for *Instrumental conditions* and *Analysis* in the Assay under *Potassium Chloride Oral Solution*, except use *Assay preparation 1* or *Assay preparation 2* instead of *Sample solution*. Calculate the quantity of KCl, in mg, in the portion of Potassium Chloride for Oral Solution taken by the formula:

$$500C(1.907)$$

in which C is as defined therein.

Potassium Chloride Extended-Release Tablets

» Potassium Chloride Extended-Release Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of potassium chloride (KCl).

Packaging and storage—Preserve in tight containers at a temperature not exceeding 30°.

Labeling—The labeling states with which *Assay preparation* the product complies only if *Assay preparation 1* is not used.

Identification—A portion of the filtrate obtained as directed for the designated *Assay preparation* in the *Assay* meets the requirements of the tests for *Potassium* (191) and for *Chloride* (191).

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 2 hours.

Standard stock solution—Prepare as directed in the *Assay* under *Potassium Chloride Oral Solution*.

Standard solutions—Prepare as directed for *Standard solutions* in the *Assay* under *Potassium Chloride Oral Solution*.

Procedure—Filter the solution under test, and dilute quantitatively with *Dissolution Medium* to obtain a test solution containing about 60 µg of potassium chloride per mL. Place 5.0 mL of the test solution in a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, mix, and proceed as directed for *Instrumental conditions* and *Analysis* in the *Assay* under *Potassium Chloride Oral Solution*. Calculate the quantity, in mg, of KCl dissolved by the formula:

$$(900F)(1.907C)$$

in which *F* is the extent of dilution of the solution under test, and the other terms are as defined therein.

Tolerances—Not more than 35% (*Q*) of the labeled amount of KCl is dissolved in 2 hours. The requirements are met if the quantities dissolved from the Tablets tested conform to the accompanying acceptance table instead of the table shown under *Dissolution* (711).

Acceptance Table

Stage	Number Tested	Acceptance Criteria
S ₁	6	Each unit is within the range $Q \pm 30\%$.
S ₂	6	Average of 12 units ($S_1 + S_2$) is within the range between $Q - 30\%$ and $Q + 35\%$, and no unit is outside the range $Q \pm 40\%$.
S ₃	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between $Q - 30\%$ and $Q + 35\%$, and not more than 2 units outside the range $Q \pm 40\%$.

Uniformity of dosage units (905): meet the requirements.

Assay—[NOTES—If necessary, first score nonsugar-coated tablets. Retain a portion of the filtrate of either *Assay preparation 1* or *Assay preparation 2* for use in the test for *Identification*.]

Standard stock solution and Standard solutions—Prepare as directed in the *Assay* under *Potassium Chloride Oral Solution*.

Assay preparation 1—Place not fewer than 20 Tablets in a suitable container with 400 mL of water, heat to boiling,

and boil for 20 minutes. Allow to cool, transfer the solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. Filter, discarding the first 20 mL of the filtrate. Transfer an accurately measured volume of the subsequent filtrate, equivalent to about 60 mg of potassium chloride, to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Assay preparation 2 (For formulations containing crystals coated with hydrophobic polymers)—Place not fewer than 20 Tablets in a 2000-mL volumetric flask. Add 1200 mL of a mixture of acetonitrile and water (1:1), and shake by mechanical means, or stir using a magnetic bar for 90 minutes. Dilute with the mixture of acetonitrile and water (1:1) to volume. Allow to stand for 90 minutes. Pass through a filter having a 0.2-µm porosity. Transfer an accurately measured volume of the filtrate, quantitatively dilute with water to obtain a solution having a concentration of about 0.06 mg per mL, and mix. [NOTE—Retain a portion of the filtrate for use in the test for *Identification*.] Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Procedure—Proceed as directed for *Instrumental conditions* and *Analysis* in the *Assay* under *Potassium Chloride Oral Solution*, except use *Assay preparation 1* or *Assay preparation 2* instead of *Sample solution*. Calculate the quantity, in mg, of potassium chloride (KCl) in each Tablet taken by the formula:

$$1.907(TC/D)$$

in which *T* is the labeled quantity, in mg, of potassium chloride in each Tablet; *D* is the concentration, in µg per mL, of potassium chloride in the designated *Assay preparation*, based on the labeled quantity per Tablet and the extent of dilution; and the other terms are as defined therein.

Potassium Chloride in Dextrose Injection

DEFINITION

Potassium Chloride in Dextrose Injection is a sterile solution of Potassium Chloride and Dextrose in Water for Injection. It contains NLT 95.0% and NMT 110.0% of the labeled amount of potassium chloride (KCl) and NLT 95.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$). It contains no antimicrobial agents.

IDENTIFICATION

A.

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

B.

The sample imparts a violet color to a nonluminous flame. Since the presence of small quantities of sodium masks the color, screen out the yellow color produced by the sodium by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

C. IDENTIFICATION TESTS—GENERAL (191), Chloride: Meets the requirements

ASSAY**• DEXTROSE**

Sample solution: Nominally 20–50 mg/mL of dextrose from Injection prepared as follows. Transfer a volume of Injection, containing 2–5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide and dilute with water to volume.

Analysis

Sample: *Sample solution*

Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* (781)).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* ($^\circ$)

l = length of the polarimeter tube (dm)

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 95.0%–105.0%

• POTASSIUM CHLORIDE

Sample solution: Transfer a volume of Injection, equivalent to 75–150 mg of potassium chloride, to a conical flask. Add water, if necessary, to bring the volume to 10 mL, and add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Indicator: Eosin Y TS

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Calculate the percentage of the labeled amount of potassium chloride (KCl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample solution* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 74.55 mg/mEq

W = nominal amount of potassium chloride in the *Sample solution* (mg)

Acceptance criteria: 95.0%–110.0%

IMPURITIES**• LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Sample solution: Nominally 2.0 mg/mL of dextrose from Injection in water

Instrumental conditions

Mode: UV

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Determine the absorbance of the *Sample solution*.

Acceptance criteria: NMT 0.25

SPECIFIC TESTS**• BACTERIAL ENDOTOXINS TEST (85):** NMT 0.5 USP Endotoxin Units/mL**• PH (791)**

Sample solution: Nominally 5% of dextrose from a portion of Injection in water

Acceptance criteria: 3.5–6.5

• OTHER REQUIREMENTS: It meets the requirements under *Injections and Implanted Drug Products* (1).**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably Type I or Type II.**• LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol/mL. The content of potassium, in mEq, is prominently displayed on the label.**• USP REFERENCE STANDARDS (11)**

USP Endotoxin RS

Potassium Chloride in Dextrose and Sodium Chloride Injection**DEFINITION**

Potassium Chloride in Dextrose and Sodium Chloride Injection is a sterile solution of Potassium Chloride, Dextrose, and Sodium Chloride in Water for Injection. It contains NLT 95.0% and NMT 110.0% of the labeled amounts of potassium (K) and chloride (Cl) and NLT 95.0% and NMT 105.0% of the labeled amounts of dextrose ($C_6H_{12}O_6 \cdot H_2O$) and sodium (Na). It contains no antimicrobial agents.

IDENTIFICATION**• A.** The sample imparts an intense yellow color to a nonluminous flame.**• B.**

Analysis: To 2 mL of Injection, add 5 mL of sodium cobaltinitrite TS.

Acceptance criteria: A yellow precipitate is formed immediately. If necessary, centrifuge the solution and examine the precipitate (presence of potassium).

• C. IDENTIFICATION TESTS—GENERAL (191), Chloride: Meets the requirements**• D.**

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water

Analysis: Add a few drops of the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

ASSAY**• CHLORIDE**

Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride, to a suitable conical flask. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Each mL of *Titrant* is equivalent to 3.545 mg of chloride (Cl). Each mg of chloride is equivalent to 0.0282 mEq of chloride (Cl).

Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = Titrant volume consumed by the *Sample solution* (mL)
 N = actual normality of the *Titrant* (mEq/mL)
 F = equivalency factor, 35.45 mg/mEq
 W = nominal amount of chloride in the *Sample solution* (mg)

Acceptance criteria: 95.0%–110.0%

• DEXTROSE

Sample solution: Nominally 20–50 mg/mL of dextrose from Injection prepared as follows. Transfer a volume of Injection, containing 2–5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide and dilute with water to volume.

Analysis

Sample: *Sample solution*

Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* (781)).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* ($^\circ$)

l = length of the polarimeter tube (dm)

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 95.0%–105.0%

• POTASSIUM AND SODIUM

Internal standard solution: 1.04 mg/mL of lithium nitrate in water prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, and dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (equivalent to 1 mEq/mL of potassium) prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride (equivalent to 1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.

Standard stock solution: 0.0391/ mg/mL of potassium (K) from the *Potassium stock solution* and 0.02299/ mg/mL of sodium (Na) from the *Sodium stock solution* prepared as follows. Transfer 0.1/ mL of the *Potassium stock solution* and 0.1/ mL of the *Sodium stock solution* to a 100-mL volumetric flask, where l and l' are the labeled amounts in (mEq/L) of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 500-mL volumetric flask, and dilute with the *Internal standard solution* to volume.

Sample solution: Transfer 5.0 mL of Injection to a 500-mL volumetric flask, and dilute with the *Internal standard solution* to volume.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: 766 nm

Sodium: 589 nm

Lithium: 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and the *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of potassium to lithium from the *Sample solution*

R_S = emission reading ratio of potassium to lithium from the *Standard solution*

C_S = concentration of potassium (K) in the *Standard stock solution* (mg/mL)

C_U = nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[NOTE—Each mg of potassium is equivalent to 0.02558 mEq of potassium.]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of sodium to lithium from the *Sample solution*

R_S = emission reading ratio of sodium to lithium from the *Standard solution*

C_S = concentration of sodium (Na) in the *Standard stock solution* (mg/mL)

C_U = nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[NOTE—Each mg of sodium is equivalent to 0.04350 mEq of sodium.]

Acceptance criteria

Potassium: 95.0%–110.0%

Sodium: 95.0%–105.0%

IMPURITIES

Delete the following:

• HEAVY METALS (231)

Sample solution: Transfer a volume of Injection as determined by the calculation below to an appropriate vessel.

Calculate the volume of Injection to use to two significant figures:

$$\text{Result} = 0.2/[(G_K L_K) + (G_D L_D) + (G_S L_S)]$$

G_K = labeled amount of potassium chloride in each 100 mL of Injection (g)

L_K = limit of heavy metals for potassium chloride, 0.001%

G_D = labeled amount of dextrose in each 100 mL of Injection (g)

L_D = limit of heavy metals for dextrose, 0.0005%

G_S = labeled amount of sodium chloride in each 100 mL of Injection (g)

L_S = limit of heavy metals for sodium chloride, 0.0005%

Adjust the volume by evaporation or addition of water to 25 mL, as necessary: it passes the test.

Acceptance criteria: Meets the requirements. (Official 1-Jan-

2018)

• **5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Sample solution: Nominally 2.0 mg/mL of dextrose from Injection in water

Instrumental conditions

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Acceptance criteria: NMT 0.25

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.5 USP Endotoxin Units/mL

• **PH (791):** 3.5–6.5

• **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products (1)*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

• **LABELING:** The label states the potassium, sodium, and chloride contents in terms of mEq in a given volume. The label also states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.

• **USP REFERENCE STANDARDS (11)**

USP Endotoxin RS

Potassium Chloride, Potassium Bicarbonate, and Potassium Citrate Effervescent Tablets for Oral Solution

» Potassium Chloride, Potassium Bicarbonate, and Potassium Citrate Effervescent Tablets for Oral Solution contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of K and Cl.

Packaging and storage—Preserve in tight containers, protected from excessive heat.

Labeling—The label states the potassium and chloride contents in terms of weight and in terms of milliequivalents. Where Tablets are packaged in individual pouches, the label instructs the user not to open until the time of use.

Identification—One Tablet dissolves in 100 mL of water with effervescence. The collected gas responds to the test for *Bicarbonate (191)*, and the resulting solution responds to the tests for *Potassium (191)*, for *Chloride (191)*, and for *Citrate (191)*.

Uniformity of dosage units (905): meet the requirements for *Weight Variation*.

Assay for potassium—

Standard stock solution and Standard solutions—Prepare as directed in the *Assay* under *Potassium Chloride Oral Solution*.

Assay preparation—Transfer 10 Potassium Chloride, Potassium Bicarbonate, and Potassium Citrate Effervescent Tablets for Oral Solution to a 2000-mL volumetric flask, dissolve in 200 mL of water, swirl until effervescence ceases, dilute with water to volume, and mix. Filter, and quantitatively dilute an accurately measured volume of the filtrate with water to obtain a solution containing 30 µg of potassium per mL. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Procedure—Proceed as directed for *Instrumental conditions* and *Analysis* in the *Assay* under *Potassium Chloride Oral Solution*, except use *Assay preparation* instead of *Sample solution*. Calculate the quantity, in mg, of potassium (K) in each Tablet taken by the formula:

$$L(C/D)$$

in which *L* is the labeled quantity, in mg, of potassium in each Tablet, *C* is the concentration, in µg per mL, of potassium in the *Assay preparation*, and *D* is the concentration, in µg per mL, of potassium in the *Assay preparation* on the basis of the labeled quantity in each Tablet and the extent of dilution.

Assay for chloride—Transfer a number of Potassium Chloride, Potassium Bicarbonate, and Potassium Citrate Effervescent Tablets for Oral Solution, equivalent to about 900 mg of chloride, to a 2000-mL volumetric flask. Add about 200 mL of water, swirl until effervescence ceases, dilute with water to volume, and mix. Transfer 25.0 mL of this solution to a 250-mL conical flask, add 50.0 mL of 0.1 N silver nitrate VS and 15 mL of nitric acid, and boil, with constant swirling, until the supernatant is colorless. Cool to room temperature, add sufficient water to make a volume of about 150 mL, add 5 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS to a permanent faint brown endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Calculate the quantity, in mg, of chloride (Cl) in each Tablet by dividing the total amount of chloride in the Tablets taken by the number of Tablets taken.

Potassium Chloride in Lactated Ringer's and Dextrose Injection

DEFINITION

Potassium Chloride in Lactated Ringer's and Dextrose Injection is a sterile solution of Calcium Chloride, Potassium Chloride, Sodium Chloride, and Sodium Lactate in Water for Injection. It contains, in each 100 mL, NLT 285.0 mg and NMT 315.0 mg of sodium [as sodium chloride (NaCl)] and anhydrous sodium lactate ($C_3H_5NaO_3$), NLT 4.90 mg and NMT 6.00 mg of calcium (Ca) [equivalent to NLT 18.0 mg and NMT 22.0 mg of calcium chloride ($CaCl_2 \cdot 2H_2O$)], and NLT 231.0 mg and NMT 261.0 mg of lactate ($C_3H_5O_3$) [equivalent to NLT 290.0 mg and NMT 330.0 mg of anhydrous sodium lactate ($C_3H_5NaO_3$)]. It contains NLT 95.0% and NMT 105.0% of the labeled amount of potassium chloride (KCl), NLT 90.0% and NMT 105.0% of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$), and NLT 90.0% and NMT 110.0% of the labeled amount of chloride (Cl) [as sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($CaCl_2 \cdot 2H_2O$)]. It contains no antimicrobial agents.

IDENTIFICATION

• **A.**

Sample solution: Nominally 50 mg/mL of dextrose from a suitable volume of Injection in water

Analysis: Add a few drops of *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

• **B. IDENTIFICATION TESTS—GENERAL (191), Calcium and Chloride:** Meets the requirements of the ammonium oxalate test for *Calcium* and the test for *Chloride*

• **C. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.

• **D. POTASSIUM:** The sample imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow

color produced by sodium by viewing through a blue filter that blocks the emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

- **E.** The retention time of the lactate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Lactate*.

ASSAY

• CALCIUM

[NOTE—Concentrations of the *Standard solution* and the *Sample solution* may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Lanthanum chloride solution: 88.45 g/L of lanthanum chloride prepared as follows. Transfer a suitable quantity of lanthanum chloride to an appropriate volumetric flask. Add 50% of the final flask volume of water. Carefully add 25% of the final flask volume of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume.

Calcium stock solution: 1 mg/mL of calcium (Ca) prepared as follows. Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of diluted hydrochloric acid, and swirl to dissolve the calcium carbonate. Dilute with water to volume.

Standard solutions: 0.010, 0.015, and 0.020 mg/mL of calcium prepared as follows. To three separate 100-mL volumetric flasks, each containing 5.0 mL of *Lanthanum chloride solution*, add 1.0, 1.5, and 2.0 mL of *Calcium stock solution*, respectively. Dilute the contents of each flask with water to volume.

Sample solution: Transfer 30.0 mL of Injection to a 100-mL volumetric flask containing 5.0 mL of *Lanthanum chloride solution*. Dilute with water to volume.

Blank: Transfer 5.0 mL of *Lanthanum chloride solution* to a 100-mL volumetric flask and dilute with water to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbances of the *Standard solutions* versus the concentration, in mg/mL, of calcium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration (C), in mg/mL, of calcium in the *Sample solution*. Calculate the quantity (mg) of calcium (Ca) in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

C = concentration of calcium in the *Sample solution*, as determined from the graph (mg/mL)

D = dilution factor of the *Sample solution*, 3.3

F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 4.90–6.00 mg of calcium (Ca) in each 100 mL

• CHLORIDE

Sample solution: Transfer 10 mL of Injection into a conical flask. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis

Sample: *Sample solution*

Titrate, with shaking, with *Titrant* to a pink endpoint.

Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = volume of *Titrant* consumed by the *Sample solution* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 35.45 mg/mEq

W = nominal amount of chloride in the *Sample solution* (mg)

Acceptance criteria: 90.0%–110.0%

• DEXTROSE

Sample solution: Transfer a volume of Injection containing 2–5 g of dextrose to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume.

Analysis

Sample: *Sample solution*

Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* (781)).

Calculate the percentage of the labeled amount of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken:

$$\text{Result} = [(100 \times a)/(l \times \alpha)] \times (1/C_U) \times (M_{r1}/M_{r2}) \times 100$$

a = observed angular rotation of the *Sample solution* (°)

l = length of the polarimeter tube (dm)

α = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

C_U = nominal concentration of dextrose in the *Sample solution* (g/100 mL)

M_{r1} = molecular weight of dextrose monohydrate, 198.17

M_{r2} = molecular weight of anhydrous dextrose, 180.16

Acceptance criteria: 90.0%–105.0%

• LACTATE

Mobile phase: Add 1 mL of formic acid and 1 mL of dicyclohexylamine per L of water.

System suitability solution: 3 mg/mL each of anhydrous sodium acetate and USP Sodium Lactate RS in water

Standard solution: 3 mg/mL of USP Sodium Lactate RS in water

Sample solution: Use the undiluted Injection.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2 between acetate and lactate, *System suitability solution*

Tailing factor: NMT 2.0 for lactate, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis**Samples:** *Standard solution* and *Sample solution*Calculate the quantity (mg) of lactate ($C_3H_5O_3$) in each 100 mL of Injection taken:

$$\text{Result} = (r_u/r_s) \times C_s \times (M_{r1}/M_{r2}) \times V$$

- r_u = peak response of lactate from the *Sample solution*
 r_s = peak response of lactate from the *Standard solution*
 C_s = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL)
 M_{r1} = molecular weight of lactate, 89.07
 M_{r2} = molecular weight of anhydrous sodium lactate, 112.06
 V = volume of the Injection, 100 mL

Acceptance criteria: 231.0–261.0 mg of lactate ($C_3H_5O_3$) in each 100 mL**• POTASSIUM****Solution A:** Suitable nonionic wetting agent (1 in 500)
Standard stock solution A: 100 µg/mL of potassium in water prepared as follows. Dissolve 190.7 mg of potassium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer the resulting solution to a 1-L volumetric flask, and dilute with water to volume.**Standard stock solution B:** 10.93 mg/mL of sodium chloride in water**Standard solutions:** 0.005, 0.010, 0.015, and 0.020 mg/mL of potassium prepared as follows. Transfer 10 mL of *Standard stock solution B* to each of four 100-mL volumetric flasks containing 10.0 mL of *Solution A*. To each flask add, respectively, 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution A*, and dilute with water to volume.**Sample solution:** Nominally 0.015 mg/mL of potassium prepared as follows. Transfer a suitable portion of Injection into an appropriate volumetric flask. Add 10% of the final flask volume of *Solution A*. Dilute with water to volume.**Blank:** Transfer 10 mL of *Standard stock solution B* to a 100-mL volumetric flask containing 10.0 mL of *Solution A*. Dilute with water to volume.**Instrumental conditions****Mode:** Atomic emission spectrophotometry**Emission wavelength:** 766 nm**Analysis****Samples:** *Standard solutions*, *Sample solution*, and *Blank*
Set a suitable flame photometer for maximum transmittance at a wavelength of 766 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of potassium. From the graph so obtained, read the percentage transmittance of the *Sample solution*.

Calculate the percentage of the labeled amount of potassium chloride (KCl) in the portion of Injection taken:

$$\text{Result} = (C/C_u) \times (M_r/A_r) \times 100$$

- C = concentration of potassium in the *Sample solution*, as determined from the graph (mg/mL)
 C_u = nominal concentration of potassium in the *Sample solution* (mg/mL)
 M_r = molecular weight of potassium chloride, 74.55
 A_r = atomic weight of potassium, 39.10

Acceptance criteria: 95.0%–105.0%**• SODIUM****Solution A:** Suitable nonionic wetting agent (1 in 500)**Standard stock solution:** 100 µg/mL of sodium in water prepared as follows. Dissolve 254.2 mg of sodium

chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer the resulting solution to a 1-L volumetric flask, and dilute with water to volume.

Standard solutions: 0.005, 0.010, 0.015, and 0.020 mg/mL of sodium prepared as follows. Transfer 10 mL of *Solution A* to each of four 100-mL volumetric flasks. To each flask add, respectively, 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution*, and dilute with water to volume.**Sample solution:** Transfer 5 mL of Injection to a 1-L volumetric flask containing 100 mL of *Solution A*. Dilute with water to volume.**Blank:** Transfer 10 mL of *Solution A* to a 100-mL volumetric flask. Dilute with water to volume.**Instrumental conditions****Mode:** Atomic emission spectrophotometry**Emission wavelength:** 589 nm**Analysis****Samples:** *Standard solutions*, *Sample solution*, and *Blank*
Set a suitable flame photometer for maximum transmittance at a wavelength of 589 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage transmittance of the other *Standard solutions*, and plot transmittances versus concentration of sodium. From the graph so obtained, read the percentage transmittance of the *Sample solution*.

Calculate the quantity (mg) of sodium (Na) in each 100 mL of Injection taken:

$$\text{Result} = C \times D \times F$$

- C = concentration of sodium in the *Sample solution*, as determined from the graph (mg/mL)
 D = dilution factor of the *Sample solution*, 200
 F = conversion factor for each 100 mL of Injection, 100 mL

Acceptance criteria: 285.0–315.0 mg of sodium (Na) in each 100 mL**IMPURITIES****Delete the following:****• HEAVY METALS (231)****Sample solution:** Transfer a volume of Injection as determined by the calculation below to an appropriate vessel. Adjust the volume by evaporation or addition of water to 25 mL, as necessary.

Calculate the volume of Injection to use to two significant figures:

$$\text{Result} = 0.2 / [(G_S L_S) + (G_K L_K) + (G_C L_C) + (G_L L_L) + (G_D L_D)]$$

- G_S = labeled amount of sodium chloride in each 100 mL of Injection (g)
 L_S = limit of heavy metals under *Sodium Chloride*
 G_K = labeled amount of potassium chloride in each 100 mL of Injection (g)
 L_K = limit of heavy metals under *Potassium Chloride*
 G_C = labeled amount of calcium chloride in each 100 mL of Injection (g)
 L_C = limit of heavy metals under *Calcium Chloride*
 G_L = labeled amount of sodium lactate in each 100 mL of Injection (g)
 L_L = limit of heavy metals under *Sodium Lactate*
 G_D = labeled amount of dextrose in each 100 mL of Injection (g)
 L_D = limit of heavy metals under *Dextrose*

Acceptance criteria: Meets the requirements. (Official 1-Jan-2018)

• **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

Sample solution: Nominally 2.0 mg/mL of dextrose ($C_6H_{12}O_6 \cdot H_2O$) from Injection in water

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 284 nm

Cell: 1 cm

Blank: Water

Analysis

Samples: *Sample solution* and *Blank*

Determine the absorbance of the *Sample solution* with a suitable spectrophotometer.

Acceptance criteria: The absorbance is NMT 0.25.

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.5 USP Endotoxin Units/mL.

• **PH (791):** 3.5–6.5

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

• **LABELING:** The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL. The label includes also the warning: "Not for use in the treatment of lactic acidosis."

• **USP REFERENCE STANDARDS (11)**

USP Endotoxin RS

USP Sodium Lactate RS

Potassium Chloride in Sodium Chloride Injection

DEFINITION

Potassium Chloride in Sodium Chloride Injection is a sterile solution of Potassium Chloride and Sodium Chloride in Water for Injection. It contains NLT 95.0% and NMT 110.0% of the labeled amounts of potassium (K) and chloride (Cl) and NLT 95.0% and NMT 105.0% of the labeled amount of sodium (Na). It contains no antimicrobial agents.

IDENTIFICATION

• **A. SODIUM:** The sample imparts an intense yellow color to a nonluminous flame.

• **B.**
Analysis: To 2 mL of Injection add 5 mL of sodium cobaltinitrite TS.

Acceptance criteria: A yellow precipitate is formed immediately. If necessary, centrifuge the solution and examine the precipitate (presence of potassium).

• **C. IDENTIFICATION TESTS—GENERAL (191), Chloride:** Meets the requirements

ASSAY

• **POTASSIUM AND SODIUM**

Internal standard solution: 1.04 mg/mL of lithium nitrate in water prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, and dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride (equivalent to 1 mEq/mL of potassium) pre-

pared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.

Sodium stock solution: 58.44 mg/mL of sodium chloride (equivalent to 1 mEq/mL of sodium) prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask. Dilute with water to volume.

Standard stock solution: 0.0391 J mg/mL of potassium (K) from the *Potassium stock solution* and 0.02299 J' mg/mL of sodium (Na) from the *Sodium stock solution* prepared as follows. Transfer 0.1 J mL of *Potassium stock solution* and 0.1 J' mL of *Sodium stock solution* to a 100-mL volumetric flask, where J and J' are the labeled amounts (in mEq/L) of potassium and sodium, respectively, in the Injection. Dilute with water to volume.

Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 500-mL volumetric flask, and dilute with the *Internal standard solution* to volume.

Sample solution: Transfer 5.0 mL of Injection to a 500-mL volumetric flask, and dilute with the *Internal standard solution* to volume.

Instrumental conditions

Mode: Flame photometer

Analytical wavelengths

Potassium: 766 nm

Sodium: 589 nm

Lithium: 671 nm

Blank: *Internal standard solution*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*
Use the *Blank* to zero the instrument. Concomitantly determine the flame emission readings for the *Standard solution* and the *Sample solution*.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of potassium to lithium from the *Sample solution*

R_S = emission reading ratio of potassium to lithium from the *Standard solution*

C_S = concentration of potassium (K) in the *Standard solution* (mg/mL)

C_U = nominal concentration of potassium (K) in the *Sample solution* (mg/mL)

[NOTE—Each mg of potassium is equivalent to 0.02558 mEq of potassium.]

Calculate the percentage of the labeled amount of sodium (Na) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = emission reading ratio of sodium to lithium from the *Sample solution*

R_S = emission reading ratio of sodium to lithium from the *Standard solution*

C_S = concentration of sodium (Na) in the *Standard solution* (mg/mL)

C_U = nominal concentration of sodium (Na) in the *Sample solution* (mg/mL)

[NOTE—Each mg of sodium is equivalent to 0.04350 mEq of sodium.]

Acceptance criteria

Potassium: 95.0%–110.0%

Sodium: 95.0%–105.0%

• **CHLORIDE**

Sample solution: Transfer a volume of Injection, equivalent to 55 mg of chloride, to a suitable conical flask.

Add 10 mL of glacial acetic acid, 75 mL of methanol, and 3 drops of eosin Y TS.

Titrimetric system**Mode:** Direct titration**Titrant:** 0.1 N silver nitrate VS**Endpoint detection:** Visual**Analysis****Sample:** Sample solutionTitrate, with shaking, with *Titrant* to a pink endpoint.

Calculate the percentage of the labeled amount of chloride (Cl) in the portion of Injection taken:

$$\text{Result} = V \times N \times (F/W) \times 100$$

V = *Titrant* volume consumed by the *Sample* solution (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 35.45 mg/mEq

W = nominal amount of chloride in the *Sample* solution (mg)

[NOTE—Each mg of chloride is equivalent to 0.0282 mEq of chloride (Cl).]

Acceptance criteria: 95.0%–110.0%**IMPURITIES****Delete the following:**• **HEAVY METALS** (231)

Sample solution: Transfer a volume of Injection as determined by the calculation below to an appropriate vessel. Adjust the volume by evaporation or addition of water to 25 mL, as necessary.

Calculate the volume of Injection to use to two significant figures.

$$\text{Result} = 0.2 / [(G_K L_K) + (G_S L_S)]$$

G_K = labeled amount of potassium chloride in each 100 mL of Injection (g)

L_K = limit of heavy metals for potassium chloride, 0.001%

G_S = labeled amount of sodium chloride in each 100 mL of Injection (g)

L_S = limit of heavy metals for sodium chloride, 0.0005%

Acceptance criteria: Meets the requirements. (Official 1-Jan-2018)

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.5 USP Endotoxin Units/mL

• **PH** (791): 3.5–6.5

• **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* (1).

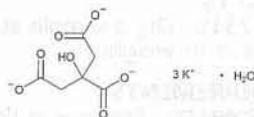
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I or Type II glass, or of a suitable plastic.

• **LABELING:** The label states the potassium, sodium, and chloride contents in terms of mEq in a given volume. The label also states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, the label alternatively may state the total osmolar concentration in mOsmol/mL.

• **USP REFERENCE STANDARDS** (11)

USP Endotoxin RS

Potassium Citrate

$C_6H_5K_3O_7 \cdot H_2O$ 324.41

$C_6H_5K_3O_7$ 306.40

1,2,3-Propanetricarboxylic acid, 2-hydroxy-, tripotassium salt, monohydrate;

Tripotassium citrate monohydrate [6100-05-6].

Anhydrous [866-84-2].

DEFINITION

Potassium Citrate contains NLT 99.0% and NMT 100.5% of potassium citrate ($C_6H_5K_3O_7$), calculated on the dried basis.

IDENTIFICATION

• **A. IDENTIFICATION TESTS—GENERAL,** *Potassium* (191) and *Citrate* (191): A 100-mg/mL solution meets the requirements.

ASSAY• **PROCEDURE**

Sample: 200 mg of Potassium Citrate

Blank: 25 mL of glacial acetic acid

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 25 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with the *Titrant* to a green endpoint. Perform a *Blank* determination.

Calculate the percentage of potassium citrate ($C_6H_5K_3O_7$) in the *Sample* taken:

$$\text{Result} = [(V_S - V_B) \times N \times F/W] \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 102.1 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES**Delete the following:**• **HEAVY METALS, Method I** (231)

Test preparation: Dissolve 2 g in 25 mL of water, and proceed as directed in the chapter, except use glacial acetic acid to adjust the pH.

Acceptance criteria: NMT 10 ppm. (Official 1-Jan-2018)

• **TARTRATE**

Sample: 1 g of Potassium Citrate

Analysis: Dissolve the *Sample* in 1.5 mL of water in a test tube, add 1 mL of 6 N acetic acid, and scratch the walls of the test tube with a glass rod.

Acceptance criteria: No crystalline precipitate is formed.

SPECIFIC TESTS• **ALKALINITY**

Sample solution: Dissolve 1 g of Potassium Citrate in 20 mL of water.

Analysis: To the *Sample solution* add 0.20 mL of 0.10 N sulfuric acid, and add 1 drop of phenolphthalein TS.

Acceptance criteria: The *Sample solution* is alkaline to litmus. No pink color is produced after the addition of phenolphthalein TS.

- **LOSS ON DRYING (731):** Dry a sample at 180° for 4 h: it loses 3.0%–6.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Citrate Extended-Release Tablets**DEFINITION**

Potassium Citrate Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of potassium citrate as monohydrate ($C_6H_5K_3O_7 \cdot H_2O$).

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Potassium (191)**

Sample solution: Powder 5 Tablets, mix with 20 mL of water, and filter.

Acceptance criteria: The filtrate meets the requirements.

- **B. IDENTIFICATION TESTS—GENERAL, Citrate (191)**

Sample: A portion of powdered Tablets containing about 50 mg of potassium citrate

Acceptance criteria: Meet the requirements

ASSAY• **PROCEDURE**

Mobile phase, Standard solution 1, and Chromatographic system: Proceed as directed under *Assay for Citric Acid/Citrate and Phosphate (345)*.

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to about 200 mg of potassium citrate monohydrate, to a 1000-mL volumetric flask, add about 300 mL of hot water, and shake by mechanical means for 15 min. Allow to cool, dilute with water to volume, and mix. Filter, discarding the first 30 mL of the filtrate. Transfer an aliquot of the clear filtrate to a suitable volumetric flask. Dilute with water and freshly prepared sodium hydroxide solution to obtain a solution containing about 20 µg/mL of citrate in 1 mM sodium hydroxide.

[NOTE—Reserve the remaining filtrate for use in *Content of Potassium*.]

Analysis

Samples: *Standard solution 1* and *Sample solution*

Calculate the percentage of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

- r_u = citrate peak area from the *Sample solution*
- r_s = citrate peak area from *Standard solution 1*
- C_s = concentration of citrate in *Standard solution 1* (µg/mL)
- C_u = nominal concentration of potassium citrate in the *Sample solution* (µg/mL)
- M_{r1} = molecular weight of potassium citrate monohydrate, 324.41
- M_{r2} = molecular weight of citrate ($C_6H_5O_7$), 189.10

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS• **CONTENT OF POTASSIUM**

Standard stock solution: 19.07 µg/mL of potassium chloride, previously dried at 105° for 2 h, in water. This solution contains 10 µg/mL of potassium.

Standard solutions: Transfer 10.0, 15.0, and 20.0 mL, respectively, to separate 100-mL volumetric flasks of *Standard stock solution*. To each flask, add 2.0 mL of sodium chloride solution (200 mg/mL) and 1.0 mL of hydrochloric acid, and dilute with water to volume. The *Standard solutions* contain 1.0, 1.5, and 2.0 µg/mL of potassium, respectively.

Sample solution: Transfer 3.0 mL of the clear filtrate, reserved from the Assay, to a 100-mL volumetric flask. Add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Instrumental conditions

(See *Atomic Absorption Spectroscopy (852)*.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Potassium emission line at 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air–acetylene

Blank: Water

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*
Plot the absorbance of the *Standard solutions* versus the concentration of potassium, in µg/mL, and draw the straight line best fitting the three plotted points. From the graph obtained, determine the concentration of potassium in the *Sample solution* (µg/mL).

Calculate the percentage of potassium (K) in the portion of Tablets taken:

$$\text{Result} = (C/C_u) \times [M_r/(3 \times A_r)] \times 100$$

- C = concentration of potassium in the *Sample solution* as determined in this test (µg/mL)
 - C_u = nominal concentration, based on the Assay value, of potassium citrate monohydrate in the *Sample solution* (µg/mL)
 - M_r = molecular weight of potassium citrate monohydrate, 324.41
 - A_r = atomic weight of potassium, 39.10
- Acceptance criteria:** 36.4%–40.2%

PERFORMANCE TESTS• **DISSOLUTION (711)****Test 1**

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 0.5, 1, and 3 h; without *Medium* replacement

[NOTE—Withdraw the same volume at each time point.]

Standard stock solution and Standard solutions: Prepare as directed in the *Content of Potassium*.

Sample solution: Filter the solution under test and dilute quantitatively with *Medium* to obtain a solution containing about 60 µg of potassium citrate per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Instrumental conditions

(See *Atomic Absorption Spectroscopy (852)*.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Potassium emission line at 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air-acetylene

Blank: Water

Analysis

Samples: Standard solutions, Sample solution, and Blank

Determine the concentration, in $\mu\text{g/mL}$, of potassium in the Sample solution at each time point.

Calculate the percentage of the labeled amount of potassium citrate monohydrate ($\text{C}_6\text{H}_5\text{K}_3\text{O}_7 \cdot \text{H}_2\text{O}$) dissolved at each time point:

At 0.5 h:

$$\text{Result}_1 = C_1 \times V \times R \times F \times 100/L$$

At 1 h:

$$\text{Result}_2 = [C_2 \times (V - V_3) + C_1 \times V_3] \times R \times F \times 100/L$$

At 3 h:

$$\text{Result}_3 = \{C_3 \times [V - 2 \times V_3] + (C_1 + C_2) \times V_3\} \times R \times F \times 100/L$$

C = as C_1 , C_2 , C_3 , concentration of potassium in the Sample solution at each time point ($\mu\text{g/mL}$)

V = volume of Medium, 900 mL

R = ratio of the molecular weight of potassium citrate monohydrate to 3 times the atomic weight of potassium, 2.765

F = dilution factor of the Sample solution

L = label claim (mg/Tablet)

V₃ = volume of sample withdrawn at each time point (mL)

Tolerances: The percentages of the labeled amount of potassium citrate monohydrate ($\text{C}_6\text{H}_5\text{K}_3\text{O}_7 \cdot \text{H}_2\text{O}$) dissolved from the Tablets are NMT 45% (Q) in 30 min, NMT 60% (Q') in 1 h, and NLT 80% (Q'') in 3 h. The requirements are met if the quantities dissolved from the Tablets tested conform to Table 1 instead of the table shown under Dissolution (711).

Table 1

Stage	Number Tested	Acceptance Criteria
S ₁	6	Each unit is within the range between $Q \pm 10\%$ and $Q' \pm 10\%$, and is NLT $Q'' + 5\%$ at the stated times.
S ₂	6	Average of 12 units ($S_1 + S_2$) is within the range between $Q \pm 10\%$ and $Q' \pm 10\%$ and is NLT Q'' ; no unit is outside the range between $Q \pm 15\%$ and $Q' \pm 15\%$, and no unit is less than $Q'' - 5\%$ at the stated times.
S ₃	12	Average of 24 units ($S_1 + S_2 + S_3$) is within the range between $Q \pm 10\%$ and $Q' \pm 10\%$ and is NLT Q'' ; NMT 1 unit is outside the range between $Q \pm 15\%$, NMT 1 unit is outside the range between $Q' \pm 15\%$, and NMT 1 unit is less than $Q'' - 5\%$ at the stated times.

Test 2: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 0.5, 1, 4, and 6 h. Replace the volume withdrawn with the equal volume of Medium preheated to $37 \pm 0.5^\circ$.

Buffer: 3.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.2.

Mobile phase: Buffer

Standard solution: Prepare a solution of USP Citric Acid RS in Medium as directed in Table 2.

Table 2

Tablet Strength (mg, as potassium citrate monohydrate)	Concentration of Citric Acid (mg/mL)
540	0.35
1080	0.70
1620	1.05

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45- μm pore size, discarding the first 5 mL of filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm \times 25-cm; 5- μm packing L1

Column temperature: 55°

Flow rate: 1.0 mL/min

Injection volume: 20 μL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Determine the concentration, in mg/mL, of potassium citrate monohydrate ($\text{C}_6\text{H}_5\text{K}_3\text{O}_7 \cdot \text{H}_2\text{O}$) in the sample withdrawn from the vessel at each time point:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

r_U = citric acid peak area from the Sample solution

r_S = citric acid peak area from the Standard solution

C_S = concentration of USP Citric Acid RS in the Standard solution (mg/mL)

M_{r1} = molecular weight of potassium citrate monohydrate ($\text{C}_6\text{H}_5\text{K}_3\text{O}_7 \cdot \text{H}_2\text{O}$), 324.41

M_{r2} = molecular weight of citric acid ($\text{C}_6\text{H}_8\text{O}_7$), 192.13

Calculate the percentage of the labeled amount of potassium citrate monohydrate ($\text{C}_6\text{H}_5\text{K}_3\text{O}_7 \cdot \text{H}_2\text{O}$) dissolved at each time point:

At 0.5 h:

$$\text{Result}_1 = C_1 \times V \times 100/L$$

At 1 h:

$$\text{Result}_2 = (C_2 \times V + C_1 \times V_3) \times 100/L$$

At 4 h:

$$\text{Result}_3 = [C_3 \times V + (C_1 + C_2) \times V_3] \times 100/L$$

At 6 h:

$$\text{Result}_4 = [C_4 \times V + (C_1 + C_2 + C_3) \times V_3] \times 100/L$$

C = as C_1 , C_2 , C_3 , C_4 concentration of potassium citrate monohydrate in the portion of sample withdrawn at the specified time point (mg/mL)

V = volume of Medium, 900 mL

L = label claim (mg/Tablet)

V₃ = volume of sample withdrawn at each time point (mL)

Tolerances: The percentage of the labeled amount of potassium citrate monohydrate ($\text{C}_6\text{H}_5\text{K}_3\text{O}_7 \cdot \text{H}_2\text{O}$) dissolved at the times specified in Table 3 conform to Acceptance Table 2 in Dissolution (711).

Table 3

Time (h)	Amount Dissolved (%)
0.5	25–50
1	40–65
4	NLT 70
6	NLT 80

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The label states the amount of potassium citrate as monohydrate ($C_6H_5K_3O_7 \cdot H_2O$) in mEq and in g/ Tablet. The label indicates the *Dissolution Test* with which the product complies.
- **USP REFERENCE STANDARDS (11)**
USP Citric Acid RS

Potassium Citrate and Citric Acid Oral Solution

DEFINITION

Potassium Citrate and Citric Acid Oral Solution is a solution of Potassium Citrate and Citric Acid in a suitable aqueous medium. In each 100 mL, it contains NLT 7.55 g and NMT 8.35 g of potassium (K), and NLT 12.18 g and NMT 13.46 g of citrate ($C_6H_5O_7$), equivalent to NLT 20.9 g and NMT 23.1 g of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$). It also contains NLT 6.34 g and NMT 7.02 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$).

[NOTE—The potassium ion content of Oral Solution is approximately 2 mEq/mL.]

IDENTIFICATION

- **A.**
Sample solution: A dilution of Oral Solution (1 in 40)
Analysis: To 2 mL of the *Sample solution* add 5 mL of sodium cobaltinitrite TS.
Acceptance criteria: A yellow precipitate is formed immediately (presence of potassium).
- **B.**
Sample solution: Oral Solution and hydrochloric acid (1:1)
Analysis: To 2 mL of the *Sample solution* add 10 mL of cobalt–uranyl acetate TS, and stir with a glass rod.
Acceptance criteria: No precipitate or turbidity forms after 15 min, and the *Sample solution* remains clear (absence of sodium).
- **C. IDENTIFICATION TESTS—GENERAL, Citrate (191)**
Sample solution: 3–5 drops of Oral Solution and 20 mL of the mixture of pyridine and acetic anhydride
Acceptance criteria: Meets the requirements

ASSAY

POTASSIUM

Sodium stock solution: 58.44 mg/mL of sodium chloride prepared as follows. Transfer 14.61 g of sodium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Potassium stock solution: 74.56 mg/mL of potassium chloride prepared as follows. Transfer 18.64 g of potassium chloride, previously dried at 105° for 2 h, to a 250-mL volumetric flask, and dilute with water to volume.

Diluent: 1.04 mg/mL of lithium nitrate prepared as follows. Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask. Add a suitable nonionic surfactant, then add water to volume. *Diluent* contains 15 mEq of lithium per 1000 mL.

Standard stock solution: 5.844 mg/mL of sodium chloride and 7.456 mg/mL of potassium chloride prepared as follows. Transfer 50 mL each of *Potassium stock solution* and *Sodium stock solution* to a 500-mL volumetric flask, and dilute with water to volume. Each mL of the *Standard stock solution* contains 0.1 mEq of sodium and 0.1 mEq of potassium.

Standard solution: 29.22 µg/mL of sodium chloride and 37.28 µg/mL of potassium chloride prepared as follows. Transfer 50 µL of the *Standard stock solution* to a 10-mL volumetric flask, and dilute with *Diluent* to volume.

Sample stock solution: Transfer a volume of Oral Solution, equivalent to about 2 g of potassium citrate monohydrate, to a 200-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 50 µL of the *Sample stock solution* to a 10-mL volumetric flask, and dilute with *Diluent* to volume.

Instrumental conditions

Mode: Flame photometer

Analytical wavelength: Wavelength of maximum emission at about 766 nm

Blank: *Diluent*

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*
Use the *Blank* to zero the instrument. Measure the emission responses for the *Standard solution* and the *Sample solution*.

Calculate the quantity, in g, of potassium (K) in each 100 mL of Oral Solution taken:

$$\text{Result} = [(r_u/r_s) \times (C_s \times F) \times (A_r/M_r)] \times D_1 \times D_2 \times 100$$

r_u = photometer reading of the *Sample solution*

r_s = photometer reading of the *Standard solution*

C_s = concentration of potassium chloride in the *Standard solution* (µg/mL)

F = conversion factor, 1 g per 10^6 µg

A_r = atomic weight of potassium, 39.10

M_r = molecular weight of potassium chloride, 74.55

D_1 = dilution factor of the *Sample stock solution*, 200 mL/V

V = volume of Oral Solution taken to prepare the *Sample stock solution* (mL)

D_2 = dilution factor used to prepare the *Sample solution*, 10 mL per 0.05 mL

Acceptance criteria: In each 100 mL, 7.55–8.35 g of potassium (K)

CITRATE

Mobile phase, Standard preparation 1, and Chromatographic system: Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate (345)*.

Sample solution: Transfer 15 mL of Oral Solution to a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate (345)*, *Assay Preparation for Citric Acid/Citrate Assay*.

Analysis

Samples: *Standard preparation 1* and *Sample solution*
Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate (345)*, *Procedure*.

Calculate the quantity, in g, of citrate ($C_6H_5O_7$) in each 100 mL of the Oral Solution taken:

$$\text{Result} = [(r_u/r_s) \times (C_s/F) \times D] - A[M_{r1}/M_{r2}] \times 100$$

r_u = peak response of citrate from the *Sample solution*

r_s = peak response of citrate from *Standard preparation 1*

C_s = concentration of citrate in *Standard preparation 1* (µg/mL)

F = conversion factor, 10^6 µg/g

D = dilution factor of the *Sample solution*

A = quantity of citric acid monohydrate in the Oral Solution determined in the Assay for Citric Acid (g/mL)

M_{r1} = molecular weight of citrate, 189.10

M_{r2} = molecular weight of citric acid monohydrate, 210.14

Acceptance criteria: In each 100 mL, 12.18–13.46 g of citrate ($C_6H_5O_7$), equivalent to NLT 20.9 g and NMT 23.1 g of potassium citrate monohydrate ($C_6H_5K_3O_7 \cdot H_2O$) and NLT 6.34 g and NMT 7.02 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$)

• CITRIC ACID

Sample solution: 15 mL of Oral Solution, diluted with water to 250 mL

Titrimetric system

Mode: Direct titration

Titrant: 0.02 N sodium hydroxide VS

Endpoint detection: Visual

Analysis

Sample: Sample solution

Transfer 5 mL of the Sample solution to a suitable flask. Add 25 mL of water and 5 drops of phenolphthalein TS. Titrate with Titrant to a pink endpoint. Record the buret reading, and calculate the volume of Titrant consumed. Each mL of Titrant is equivalent to 1.401 mg of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$).

Acceptance criteria: 6.34–7.02 g of citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) in each 100 mL of Oral Solution

SPECIFIC TESTS

• **PH (791):** 4.9–5.4

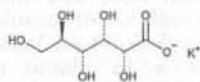
ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Citric Acid RS

Potassium Gluconate



$C_6H_{11}KO_7$ 234.25

$C_6H_{11}KO_7 \cdot H_2O$ 252.26

D-Gluconic acid, monopotassium salt;

Monopotassium D-gluconate.

Anhydrous [299–27–4].

Monohydrate [35398–15–3].

DEFINITION

Potassium Gluconate is anhydrous or contains one molecule of water of hydration. It contains NLT 97.0% and NMT 103.0% of anhydrous potassium gluconate ($C_6H_{11}KO_7$), calculated on the dried basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION (197M)**

Delete the following:

• **B. IDENTIFICATION TESTS—GENERAL, Potassium (191):**

Meets the requirements of the flame test. ▲USP40

Add the following:

• **B.** Potassium Gluconate imparts a violet color to a nonluminous flame. Because the presence of small quantities of sodium masks the color, screen out the yellow

color produced by sodium by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium).

[NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.] ▲USP40

• C. THIN-LAYER CHROMATOGRAPHY

Standard solution: 10 mg/mL of USP Potassium Gluconate RS

Sample solution: 10 mg/mL of Potassium Gluconate

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 μ L

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: Standard solution and Sample solution

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the Spray reagent. Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot of the Sample solution corresponds in color, size, and R_f to that of the Standard solution.

ASSAY

• PROCEDURE

Standard stock solution: Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, to a 1000-mL volumetric flask, add sufficient water to dissolve, and dilute with water to volume. Transfer 100.0 mL of this solution to a 1000-mL volumetric flask, and dilute with water to volume. This solution contains 10 μ g/mL of potassium (equivalent to 19.07 μ g/mL of potassium chloride).

Standard solutions: Transfer 10.0, 15.0, and 20.0 mL of Standard stock solution to separate 100-mL volumetric flasks. Add 2.0 mL of a 200-mg/mL sodium chloride solution and 1.0 mL of hydrochloric acid to each flask. Dilute with water to volume and mix. The Standard solutions contain 1.0, 1.5, and 2.0 μ g/mL of potassium, respectively.

Sample stock solution: 0.18 mg/mL of Potassium Gluconate in water. Filter the solution.

Sample solution: Transfer 5.0 mL of the filtrate from the Sample stock solution to a 100-mL volumetric flask. Add 2.0 mL of a 200-mg/mL sodium chloride solution and 1.0 mL of hydrochloric acid, and dilute with water to volume.

Blank: Water

Instrumental conditions

(See Atomic Absorption Spectroscopy (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: Standard solutions and Sample solution

Determine the absorbances of the Standard solutions and the Sample solution. Plot the absorbances of the Standard solutions versus their concentrations, in μ g/mL, of potassium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C_x , in μ g/mL, of potassium in the Sample solution.

Calculate the percentage of potassium gluconate ($C_6H_{11}KO_7$) in the portion of Potassium Gluconate taken:

$$\text{Result} = (C_K/C_U) \times (M_r/A_r) \times 100$$

C_K = determined concentration of potassium in the Sample solution ($\mu\text{g/mL}$)

C_U = concentration of Potassium Gluconate in the Sample solution ($\mu\text{g/mL}$)

M_r = molecular weight of potassium gluconate, 234.25

A_r = atomic weight of potassium, 39.10

Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

Delete the following:

• HEAVY METALS, Method I (231)

Test preparation: 1.0 g in 10 mL of water. Add 6 mL of 3 N hydrochloric acid, and dilute with water to 25 mL.

Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

• REDUCING SUBSTANCES

Sample: 1.0 g of Potassium Gluconate

Blank: 10 mL of water

Titrimetric system

(See *Titrimetry* (541).)

Mode: Residual titration

Titrant: Iodine

Back-titrant: Sodium thiosulfate

Endpoint detection: Visual

Analysis: Transfer the Sample to a 250-mL conical flask, dissolve in 10 mL of water, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid, and titrate with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the blank determination.

Calculate the percentage of reducing substances (as dextrose) in the portion of the Sample taken:

$$\text{Result} = \{[(V_B - V_S) \times N_A \times F]/W\} \times 100$$

V_B = Back-titrant volume consumed by the Blank (mL)

V_S = Back-titrant volume consumed by the Sample (mL)

N_A = actual Back-titrant normality (mEq/mL)

F = equivalency factor, 27 mg/mEq

W = Sample weight (mg)

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

• LOSS ON DRYING (731)

Analysis: Dry under vacuum at 105° for 4 h.

Acceptance criteria

Anhydrous: NMT 3.0%

Monohydrate: 6.0%–7.5%

ADDITIONAL REQUIREMENTS

• PACKAGING AND STORAGE: Preserve in tight containers.

• LABELING: Label it to indicate whether it is the anhydrous or the monohydrate form.

• USP REFERENCE STANDARDS (11)

USP Potassium Gluconate RS

Potassium Gluconate Oral Solution

DEFINITION

Potassium Gluconate Oral Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of potassium gluconate ($C_6H_{11}KO_7$).

IDENTIFICATION

Delete the following:

• A. IDENTIFICATION TESTS—GENERAL, Potassium (191):

Meets the requirements of the flame test. ^{USP40}

Add the following:

• A. Oral Solution imparts a violet color to a nonluminous flame. The presence of small quantities of sodium can mask the color unless the yellow color produced by sodium is screened out by viewing through a blue filter that blocks emission at 589 nm (sodium), but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.] ^{USP40}

• B.

Analysis: Evaporate 5 mL on a steam bath to dryness.

Acceptance criteria: A mineral oil dispersion of the residue exhibits an IR absorption maximum in the spectral region of 6.2–6.25 μm (carboxylic acid salt).

ASSAY

• PROCEDURE

Standard stock solution: Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, to a 1000-mL volumetric flask, add sufficient water to dissolve, and dilute with water to volume. Transfer 100.0 mL of this solution to a 1000-mL volumetric flask, and dilute with water to volume. This solution contains 10 $\mu\text{g/mL}$ of potassium (equivalent to 19.07 $\mu\text{g/mL}$ of potassium chloride).

Standard solutions: Transfer 10.0, 15.0, and 20.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks. To each flask add 2.0 mL of a 200-mg/mL sodium chloride solution and 1.0 mL of hydrochloric acid. Dilute with water to volume, and mix. The *Standard solutions* contain 1.0, 1.5, and 2.0 $\mu\text{g/mL}$ of potassium, respectively.

Sample solution: Transfer an accurately measured volume of Oral Solution, equivalent to 1.8 g of potassium gluconate, to a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of the solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 2.0 mL of sodium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Blank: Water

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air-acetylene

Analysis

Samples: *Standard solutions* and *Sample solution*

Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the

Standard solutions versus their concentrations, in $\mu\text{g/mL}$, of potassium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C_K , in $\mu\text{g/mL}$, of potassium in the *Sample solution*.

Calculate the percentage of the labeled amount of potassium gluconate ($\text{C}_6\text{H}_{11}\text{KO}_7$) in the portion of Oral Solution taken:

$$\text{Result} = (C_K/C_U) \times (M_r/A_r) \times 100$$

C_K = determined concentration of potassium in the *Sample solution*, ($\mu\text{g/mL}$)

C_U = nominal concentration of potassium gluconate in the *Sample solution* ($\mu\text{g/mL}$)

M_r = molecular weight of potassium gluconate, 234.25

A_r = atomic weight of potassium, 39.10

Acceptance criteria: 95.0%–105.0%

SPECIFIC TESTS

- **ALCOHOL DETERMINATION** (611), *Method II—Distillation*
Method: 4.5%–5.5% of alcohol ($\text{C}_2\text{H}_5\text{OH}$)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers.

Potassium Gluconate Tablets

DEFINITION

Potassium Gluconate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of potassium gluconate ($\text{C}_6\text{H}_{11}\text{KO}_7$).

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M): The IR absorption spectrum of potassium gluconate extracted from finely powdered Tablets exhibits maxima only at the same wavelengths as those of a similar preparation of USP Potassium Gluconate RS.

Delete the following:

- **B. IDENTIFICATION TESTS—GENERAL**, *Potassium* (191)
Sample solution: Triturate a portion of powdered Tablets with a few mL of water, and filter.
Acceptance criteria: The filtrate meets the requirements of the flame test. ▲USP40

Add the following:

- **B.**
Sample solution: Triturate a portion of powdered Tablets with a few milliliters of water, and filter.
Acceptance criteria: The *Sample solution* imparts a violet color to a nonluminous flame; the presence of small quantities of sodium masks the color, unless the yellow color produced by sodium is screened out by viewing through a blue filter that blocks the emission at 589 nm (sodium); it is transparent to the emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.] ▲USP40

ASSAY

PROCEDURE

- **Standard stock solution**: 19.07 $\mu\text{g/mL}$ of potassium chloride in water (equivalent to 10 $\mu\text{g/mL}$ of potas-

sium), prepared from potassium chloride previously dried at 105° for 2 h

Standard solutions: 1.0, 1.5, and 2.0 $\mu\text{g/mL}$ of potassium from suitably diluted *Standard stock solution*, in a solution containing 4 mg/mL of sodium chloride and 1 mL of hydrochloric acid per 100 mL

Sample stock solution: Filtered water solution containing 0.18 mg/mL of potassium gluconate from NLT 20 finely powdered Tablets

Sample solution: Transfer 5.0 mL of *Sample stock solution* to a 100-mL volumetric flask. Add 2.0 mL of a 200-mg/mL sodium chloride solution and 1.0 mL of hydrochloric acid, and dilute with water to volume.

Blank: Water

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solutions* and *Sample solution*

Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus their concentrations, in $\mu\text{g/mL}$, of potassium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C , in $\mu\text{g/mL}$, of potassium in the *Sample solution*.

Calculate the percentage of the labeled amount of potassium gluconate ($\text{C}_6\text{H}_{11}\text{KO}_7$) in the portion of Tablets taken:

$$\text{Result} = (C/C_U) \times (M_r/A_r) \times 100$$

C = determined concentration of potassium in the *Sample solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of potassium gluconate in the *Sample solution* ($\mu\text{g/mL}$)

M_r = molecular weight of potassium gluconate, 234.25

A_r = atomic weight of potassium, 39.10

Acceptance criteria: 95.0%–105.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 100 rpm

Time: 45 min

Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium* if necessary

Analysis: Proceed as directed in the *Assay*.

Calculate the percentage of the labeled amount of potassium gluconate ($\text{C}_6\text{H}_{11}\text{KO}_7$) dissolved:

$$\text{Result} = (C \times D \times V/L) \times (M_r/A_r) \times 100$$

C = determined concentration of potassium in the *Sample solution* (mg/mL)

D = dilution factor for the *Sample solution*

V = volume of *Medium*, 900 mL

L = label claim (mg/Tablet)

M_r = molecular weight of potassium gluconate, 234.25

A_r = atomic weight of potassium, 39.10

Tolerances: NLT 75% (Q) of the labeled amount of potassium gluconate ($\text{C}_6\text{H}_{11}\text{KO}_7$) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Potassium Gluconate RS

Potassium Gluconate and Potassium Chloride Oral Solution

» Potassium Gluconate and Potassium Chloride Oral Solution is a solution of Potassium Gluconate and Potassium Chloride in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of potassium (K) and chloride (Cl).

Packaging and storage—Preserve in tight containers.

Labeling—Label it to state the potassium and chloride contents in terms of milliequivalents of each in a given volume of Oral Solution.

Identification—

A: To 2 mL of a dilution of Oral Solution (1 in 40) add 5 mL of sodium cobaltinitrite TS: a yellow precipitate is formed immediately (*presence of potassium*).

B: Evaporate 5 mL on a steam bath to dryness: a mineral oil dispersion of the residue so obtained exhibits an IR absorption maximum in the spectral region between 6.2 and 6.25 μm (*carboxylic acid salt*).

C: It responds to the tests for Chloride (191).

Assay for potassium—

Potassium stock solution—Dissolve in water 0.9535 g of potassium chloride, previously dried at 105° for 2 hours. Transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. This solution contains 1000 μg of potassium per mL.

Standard preparations—To separate 200-mL volumetric flasks transfer 19.0 mL and 25.0 mL, respectively, of *Potassium stock solution*, dilute with water to volume, and mix. The *Standard preparations* contain 95.0 μg and 125.0 μg of potassium per mL, respectively.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 782 mg (20 mEq) of potassium, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of the resulting solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the resonance line of 766.5 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a potassium hollow-cathode lamp and an air-acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in μg per mL, of potassium. From the graph so obtained, determine the concentration, C, in μg per mL, of potassium in the *Assay preparation*. Calculate the quantity, in mg, of potassium in each mL of the Oral Solution taken by the formula:

$$(50/7)(C/V)$$

in which V is the volume, in mL, of Oral Solution taken. Each mg of potassium is equivalent to 0.02558 mEq.

Assay for chloride—

Ionic strength adjusting solution—Use 5 M sodium nitrate.

Procedure—Transfer an accurately measured volume of Oral Solution, equivalent to about 100 mg (2.8 mEq) of

chloride, to a suitable beaker. Add 2.0 mL of *Ionic strength adjusting solution* and water to make about 100 mL, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-sulfide specific ion-selective electrode and a double-junction reference electrode containing potassium nitrate solution (1 in 10). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Each mg of chloride is equivalent to 0.0282 mEq of Cl.

Potassium Gluconate and Potassium Chloride for Oral Solution

» Potassium Gluconate and Potassium Chloride for Oral Solution is a dry mixture of Potassium Gluconate and Potassium Chloride and one or more suitable colors, diluents, and flavors. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of potassium (K) and chloride (Cl).

Packaging and storage—Preserve in tight containers.

Labeling—Label it to state the potassium and chloride contents in terms of milliequivalents. Where packaged in unit-dose pouches, the label instructs the user not to open until the time of use.

Identification—

A: Ignite about 200 mg at a temperature not above 600°, in order to remove all organic matter, cool, dissolve the residue in 10 mL of water, and filter: the filtrate responds to the tests for Potassium (191) and for Chloride (191).

B: A mineral oil dispersion of it exhibits an IR absorption maximum in the spectral region between 6.2 and 6.25 μm (*carboxylic acid salt*).

Minimum fill (755): meets the requirements.

Assay for potassium—

Potassium stock solution—Dissolve in water 0.9535 g of potassium chloride, previously dried at 105° for 2 hours. Transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. This solution contains 1000 μg of potassium per mL.

Standard preparations—To separate 200-mL volumetric flasks transfer 19.0 mL and 25.0 mL, respectively, of the *Potassium stock solution*, dilute with water to volume, and mix. The *Standard preparations* contain 95.0 μg and 125.0 μg of potassium per mL, respectively.

Assay preparation 1 (where it is packaged in unit-dose containers)—Weigh and mix the contents of not less than 20 containers of Potassium Gluconate and Potassium Chloride for Oral Solution. Transfer an accurately weighed portion of the powder, equivalent to about 782 mg (20 mEq) of potassium, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this stock solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Assay preparation 2 (where it is packaged in multiple-unit containers)—Transfer an accurately weighed portion of Potassium Gluconate and Potassium Chloride for Oral Solution, equivalent to about 780 mg (20 mEq) of potassium, to a 100-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. Transfer 7.0 mL of this stock solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the resonance line of 766.5 nm, with a suitable atomic absorp-

tion spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a potassium hollow-cathode lamp and an air-acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in μg per mL, of potassium. From the graph so obtained, determine the concentration, C , in μg per mL, of potassium in the *Assay preparation*. Calculate the quantity, in mg, of potassium in the portion of Potassium Gluconate and Potassium Chloride for Oral Solution taken by the formula:

$$50C / 7.$$

Each mg of potassium is equivalent to 0.02558 mEq.

Assay for chloride—

Ionic strength adjusting solution—Use 5 M sodium nitrate.

Assay preparation 1 (where it is packaged in unit-dose containers)—Weigh and mix the contents of not less than 20 containers of Potassium Gluconate and Potassium Chloride for Oral Solution. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg (2.8 mEq) of chloride, to a suitable beaker.

Assay preparation 2 (where it is packaged in multiple-unit containers)—Transfer an accurately weighed portion of Potassium Gluconate and Potassium Chloride for Oral Solution, equivalent to about 100 mg (2.8 mEq) of chloride, to a suitable beaker.

Procedure—Add 2.0 mL of *Ionic strength adjusting solution* to *Assay preparation 1* or *Assay preparation 2*, add water to make about 100 mL, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-sulfide specific ion-selective electrode and a double-junction reference electrode containing potassium nitrate solution (1 in 10). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Each mg of chloride is equivalent to 0.0282 mEq of Cl.

Potassium Gluconate and Potassium Citrate Oral Solution

» Potassium Gluconate and Potassium Citrate Oral Solution is a solution of Potassium Gluconate and Potassium Citrate in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of potassium (K).

Packaging and storage—Preserve in tight containers.

Labeling—Label it to state the potassium content in terms of milliequivalents in a given volume of Oral Solution.

Identification—

A: To 2 mL of a dilution of Oral Solution (1 in 40) add 5 mL of sodium cobaltinitrite TS: a yellow precipitate is formed immediately (*presence of potassium*).

B: It responds to the test for *Citrate* (191), 3 to 5 drops of Oral Solution and 20 mL of the mixture of pyridine and acetic anhydride being used.

Assay for potassium—

Potassium stock solution—Dissolve in water 0.9535 g of potassium chloride, previously dried at 105° for 2 hours. Transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. This solution contains 1000 μg of potassium per mL.

Standard preparations—To separate 200-mL volumetric flasks transfer 19.0 mL and 25.0 mL, respectively, of *Potassium stock solution*, dilute with water to volume, and mix.

The *Standard preparations* contain 95.0 μg and 125.0 μg of potassium per mL, respectively.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 782 mg (20 mEq) of potassium, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the resonance line of 766.5 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a potassium hollow-cathode lamp and an air-acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in μg per mL, of potassium. From the graph so obtained, determine the concentration, C , in μg per mL, of potassium in the *Assay preparation*. Calculate the quantity, in mg, of potassium in each mL of the Oral Solution taken by the formula:

$$(50 / 7)(C / V)$$

in which V is the volume, in mL, of Oral Solution taken. Each mg of potassium is equivalent to 0.02558 mEq.

Potassium Gluconate, Potassium Citrate, and Ammonium Chloride Oral Solution

» Potassium Gluconate, Potassium Citrate, and Ammonium Chloride Oral Solution is a solution of Potassium Gluconate, Potassium Citrate, and Ammonium Chloride in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of potassium (K) and chloride (Cl).

Packaging and storage—Preserve in tight containers.

Labeling—Label it to state the potassium and chloride contents in terms of milliequivalents of each in a given volume of Oral Solution.

Identification—

A: To 2 mL of a dilution of Oral Solution (1 in 40) add 5 mL of sodium cobaltinitrite TS: a yellow precipitate is formed immediately (*presence of potassium*).

B: It responds to the test for *Citrate* (191), 3 to 5 drops of Oral Solution and 20 mL of the mixture of pyridine and acetic anhydride being used.

C: It responds to the tests for *Ammonium* (191) and for *Chloride* (191).

Assay for potassium—

Potassium stock solution—Dissolve in water 0.9535 g of potassium chloride, previously dried at 105° for 2 hours. Transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. This solution contains 1000 μg of potassium per mL.

Standard preparations—To separate 200-mL volumetric flasks transfer 19.0 mL and 25.0 mL, respectively, of the *Potassium stock solution*, dilute with water to volume, and mix. The *Standard preparations* contain 95.0 μg and 125.0 μg of potassium per mL, respectively.

Assay preparation—Transfer an accurately measured volume of Oral Solution, equivalent to about 782 mg (20 mEq) of potassium, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 7.0 mL of this solution

to a 500-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the resonance line of 766.5 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a potassium hollow-cathode lamp and an air-acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of potassium. From the graph so obtained, determine the concentration, C , in µg per mL, of potassium in the *Assay preparation*. Calculate the quantity, in mg, of potassium in each mL of the Oral Solution taken by the formula:

$$(50 / 7)(C / V)$$

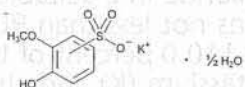
in which V is the volume, in mL, of Oral Solution taken. Each mg of potassium is equivalent to 0.02558 mEq.

Assay for chloride—

Ionic strength adjusting solution—Use 5 M sodium nitrate.

Procedure—Transfer an accurately measured volume of Oral Solution, equivalent to about 100 mg (2.8 mEq) of chloride, to a suitable beaker. Add 2.0 mL of *Ionic strength adjusting solution* and water to make about 100 mL, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-sulfide specific ion-selective electrode and a double-junction reference electrode containing potassium nitrate solution (1 in 10). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Each mg of chloride is equivalent to 0.0282 mEq of Cl.

Potassium Guaiacolsulfonate



$C_7H_7KO_5S \cdot \frac{1}{2}H_2O$	251.30
Benzenesulfonic acid, hydroxymethoxy-, monopotassium salt, hemihydrate;	
Potassium hydroxymethoxybenzenesulfonate hemihydrate [78247-49-1].	
Anhydrous	242.30

DEFINITION

Potassium Guaiacolsulfonate contains NLT 98.0% and NMT 102.0% of potassium guaiacolsulfonate ($C_7H_7KO_5S$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M): 7 µm–13 µm, previously dried at 105° for 18 h
- **B. ULTRAVIOLET ABSORPTION** (197U)
Solution: 50 µg/mL, prepared as directed in the Assay
Acceptance criteria: Meets the requirements
- **C. IDENTIFICATION TESTS—GENERAL, Potassium** (191)
Sample: A 1 in 10 solution
Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Sample stock solution: Transfer about 250 mg of Potassium Guaiacolsulfonate to a 500-mL volumetric flask. Dissolve in 400 mL of water, dilute with water to volume, and mix.

Sample solution: Dilute 10.0 mL of *Sample stock solution* with pH 7.0 phosphate buffer to 100.0 mL, and mix.

Standard solution: About 50 µg/mL of USP Potassium Guaiacolsulfonate RS in the same diluent used for the *Sample solution*

Instrumental conditions

Analytical wavelength: Maximum absorbance at about 279 nm

Cell: 1 cm

Blank: A 1 in 10 mixture of water and pH 7.0 phosphate buffer

Analysis

Samples: *Sample solution* and *Standard solution*

Concomitantly determine the absorbances of the *Sample solution* and *Standard solution*, and calculate the percentage of potassium guaiacolsulfonate ($C_7H_7KO_5S$) in the portion of Potassium Guaiacolsulfonate taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Potassium Guaiacolsulfonate RS in the *Standard solution* (µg/mL)

C_U = concentration of Potassium Guaiacolsulfonate in the *Sample solution* (µg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **SELENIUM** (291): 0.003%

• SULFATE

Analysis: To 10 mL of a solution (1 in 20) add 5 drops of barium chloride TS, and acidify with hydrochloric acid.

Acceptance criteria: No turbidity is produced in 1 min.

SPECIFIC TESTS

- **WATER DETERMINATION, Method I** (921): 3.0%–6.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
USP Potassium Guaiacolsulfonate RS

Potassium Iodide

KI	166.00
Potassium iodide [7681-11-0].	

DEFINITION

Potassium Iodide contains NLT 99.0% and NMT 101.5% of KI, calculated on the dried basis.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Potassium** (191): Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL, Iodide** (191): Meets the requirements

ASSAY

• PROCEDURE

Sample solution: Dissolve 500 mg of Potassium Iodide in 10 mL of water.

Analysis: Add 35 mL of hydrochloric acid to the *Sample solution*, and titrate with 0.05 M potassium iodate VS until the dark brown solution that is produced becomes pale brown. Add 2–3 drops of amaranth TS, and continue the titration slowly until the red color just changes to yellow. Each mL of 0.05 M potassium iodate is equivalent to 16.60 mg of KI.

Acceptance criteria: 99.0%–101.5% on the dried basis

IMPURITIES

Delete the following:

- **HEAVY METALS (231)**
Sample solution: Dissolve 2.0 g in 25 mL of water.
Acceptance criteria: NMT 10 ppm (Official 1-Jan-2018)
- **IODATE**
Iodate solution: Dilute 1 mL of potassium iodate solution (1 in 2500) with water to 100 mL.
Standard solution: Dissolve 100 mg of Potassium iodide in ammonia- and carbon dioxide-free water, and add 1 mL of Iodate solution to obtain 10 mL of solution. Transfer to a color-comparison tube, add 1 mL of starch TS and 0.25 mL of 1.0 N sulfuric acid, and mix.
Sample solution: Dissolve 1.1 g in sufficient ammonia- and carbon dioxide-free water to obtain 10 mL of solution. Transfer to a color-comparison tube, add 1 mL of starch TS and 0.25 mL of 1.0 N sulfuric acid, and mix.
Acceptance criteria: Any color produced in the Sample solution does not exceed that produced in the Standard solution (NMT 4 µg/g).
- **LIMIT OF NITRATE, NITRITE, AND AMMONIA**
Sample solution: Dissolve 1 g in 5 mL of water.
Analysis: To the Sample solution contained in a test tube of 40-mL capacity add 5 mL of 1 N sodium hydroxide and 200 mg of aluminum wire. Insert a pledget of purified cotton in the upper portion of the test tube, and place a piece of moistened red litmus paper over the mouth of the tube. Heat the test tube and its contents in a steam bath for 15 min.
Acceptance criteria: No blue coloration of the paper is discernible.
- **THIOSULFATE AND BARIUM**
Sample solution: Dissolve 0.5 g in 10 mL of ammonia- and carbon dioxide-free water.
Analysis: Add 2 drops of 2 N sulfuric acid.
Acceptance criteria: No turbidity develops within 1 min.

SPECIFIC TESTS

- **ALKALINITY**
Sample solution: Dissolve 1.0 g of Potassium Iodide in 10 mL of water.
Analysis: Add 0.1 mL of 0.1 N sulfuric acid and 1 drop of phenolphthalein TS to the Sample solution.
Acceptance criteria: No color is produced.
- **LOSS ON DRYING (731)**: Dry a sample at 105° for 4 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in well-closed containers.

Potassium Iodide Oral Solution

DEFINITION

Potassium Iodide Oral Solution contains NLT 94.0% and NMT 106.0% of the labeled amount of potassium iodide (KI). [NOTE—If Potassium Iodide Oral Solution is not to be used within a short time, add 0.5 mg of sodium thiosulfate for each g of KI. Products that have data to demonstrate acceptable stability without the addition of thiosulfate are exempt from this requirement. Crystals of potassium iodide may form in Potassium Iodide Oral Solution under normal conditions of storage, especially if refrigerated.]

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL**, Potassium (191) and Iodide (191): Meets the requirements

ASSAY

- **PROCEDURE**
Sample solution: 50 mg/mL of potassium iodide from Oral Solution in water
Analysis: Transfer 10.0 mL of the Sample solution to a 150-mL beaker, and add 40 mL of water, 25 mL of alcohol, and 1.0 mL of 1 N nitric acid. Titrate with 0.1 N silver nitrate VS, using a silver indicator electrode and an appropriate reference electrode. Perform a blank determination (see *Titrimetry* (541)). Each mL of 0.1 N silver nitrate is equivalent to 16.60 mg of KI.
Acceptance criteria: 94.0%–106.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905)**
For Oral Solution packaged in single-unit containers: Meets the requirements
- **DELIVERABLE VOLUME (698)**
For Oral Solution packaged in multiple-unit containers: Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers.

Potassium Iodide Tablets

DEFINITION

Potassium Iodide Tablets contain NLT 94.0% and NMT 106.0% of the labeled amount of potassium iodide (KI) for Tablets of 300 mg or more, and NLT 92.5% and NMT 107.5% for Tablets of less than 300 mg.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL**, Potassium (191) and Iodide (191): A filtered solution of powdered Tablets meets the requirements.

ASSAY

- **PROCEDURE**
Sample solution: Transfer an equivalent to 1.2 g of potassium iodide, from finely powdered Tablets (NLT 20), to a 250-mL volumetric flask. Add 100 mL of water, shake for 20 min, and dilute with water to volume. Filter through paper, discarding the first 20 mL of the filtrate.
Analysis: Transfer 100.0 mL of the filtrate, 25 mL of alcohol, and 1.0 mL of 1 N nitric acid to a 200-mL beaker. Titrate with 0.1 N silver nitrate VS, using a silver indicator electrode and an appropriate reference electrode. Perform a blank determination (see *Titrimetry* (541)). Each mL of 0.1 N silver nitrate is equivalent to 16.60 mg of KI.
Acceptance criteria: 94.0%–106.0% of the labeled amount of potassium iodide (KI) for Tablets of 300 mg or more, and 92.5%–107.5% for Tablets of less than 300 mg

PERFORMANCE TESTS

- **DISSOLUTION (711)**
For Uncoated Tablets
Medium: Water; 900 mL
Apparatus 2: 50 rpm
Time: 15 min
Detector: UV 227 nm
Standard solution: Potassium iodide in Medium
Sample solution: Sample per Dissolution (711). Dilute with Medium to a concentration that is similar to the Standard solution.

Tolerances: NLT 75% (Q) of the labeled amount of potassium iodide (KI) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Iodide Delayed-Release Tablets

DEFINITION

Potassium Iodide Delayed-Release Tablets contain NLT 94.0% and NMT 106.0% of the labeled amount of potassium iodide (KI) for Tablets of 300 mg or more, and NLT 92.5% and NMT 107.5% for Tablets of less than 300 mg.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Potassium (191) and Iodide (191):** A filtered solution of powdered Tablets meets the requirements.

ASSAY

• PROCEDURE

Sample solution: Transfer an equivalent to 1.2 g of potassium iodide, from finely powdered Tablets (NLT 20), to a 250-mL volumetric flask. Add 100 mL of water, shake for 20 min, and dilute with water to volume. Filter through paper, discarding the first 20 mL of the filtrate. Transfer 100.0 mL of the filtrate, 25 mL of alcohol, and 1.0 mL of 1 N nitric acid to a 200-mL beaker.

Analysis: Transfer 100.0 mL of the filtrate, 25 mL of alcohol, and 1.0 mL of 1 N nitric acid to a 200-mL beaker. Titrate with 0.1 N silver nitrate VS, using a silver indicator electrode and an appropriate reference electrode. Perform a blank determination (see *Titrimetry* (541)). Each mL of 0.1 N silver nitrate is equivalent to 16.60 mg of KI.

Acceptance criteria: 94.0%–106.0% of the labeled amount of potassium iodide (KI) for Tablets of 300 mg or more, and 92.5%–107.5% for Tablets of less than 300 mg

PERFORMANCE TESTS

• DISINTEGRATION (701)

Analysis: Proceed as directed for *Delayed-Release (Enteric-Coated) Tablets*.

Acceptance criteria: The Tablets do not disintegrate after 1 h of agitation in simulated gastric fluid TS, but they disintegrate within 90 min in simulated intestinal fluid TS.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Nitrate

KNO_3 101.10
Potassium nitrate [7757-79-1].

DEFINITION

Potassium Nitrate contains NLT 99.0% and NMT 100.5% of KNO_3 .

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Potassium (191):** Meets the requirements

- **B. IDENTIFICATION TESTS—GENERAL, Nitrate (191):** Meets the requirements

ASSAY

• PROCEDURE

[NOTE—Use water that is free of carbon dioxide and ammonia.]

Cation-exchange column: Transfer strongly acidic styrene-divinylbenzene cation-exchange resin (16- to 50-mesh) to a 2-cm diameter chromatographic column to a depth of about 20 cm.

Sample solution: 4 mg/mL of Potassium Nitrate in water

Analysis: Pass 100 mL of *Sample solution* through the *Cation-exchange column* at a rate of 5 mL/min, and collect the eluate in a 500-mL conical flask. Wash the resin in the column with water at a rate of 10 mL/min, collecting the eluate in the conical flask. Add 0.15 mL of phenolphthalein TS to the flask, and after 5 min titrate with 0.1 N sodium hydroxide VS to a pink endpoint. Continue collecting the wash from the column, and continue titrating, if necessary, until a 50-mL increment of eluate requires no further addition of sodium hydroxide. Each mL of 0.1 N sodium hydroxide is equivalent to 10.11 mg of KNO_3 .

Acceptance criteria: 99.0%–100.5%

IMPURITIES

- **CHLORIDE AND SULFATE, Chloride (221):** A 500-mg portion shows no more chloride than corresponds to 0.21 mL of 0.020 N hydrochloric acid (0.03%).

- **CHLORIDE AND SULFATE, Sulfate (221)**

Sample solution: 100 mg of Potassium Nitrate in 10 mL of water. Add 15 mL of 6 N hydrochloric acid, and evaporate to dryness on a steam bath. To the residue add 7 mL of 6 N hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 35 mL of water and, if necessary, neutralize with hydrochloric acid using a litmus paper indicator. Filter, if necessary, to obtain a clear *Sample solution*.

Acceptance criteria: The *Sample solution* shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.1%).

- **ARSENIC, Method I (211):** NMT 3 ppm

- **LEAD (251)**

Sample solution: 500 mg in 20 mL of water

Acceptance criteria: NMT 10 ppm

- **IRON (241):** NMT 10 ppm

Delete the following:

- **HEAVY METALS, Method I (231):** NMT 20 ppm (Official 1-Jan-2018)

• LIMIT OF SODIUM

Standard stock solution: [NOTE—Sodium chloride is previously dried at 105° for 2 h.] 2.542 µg/mL of sodium chloride in water. This solution contains 1.0 µg/mL of sodium.

Sample stock solution: 2 mg/mL of Potassium Nitrate. [NOTE—The concentration of potassium nitrate in this solution may be modified by using a different quantity or by further dilution to bring the absorption response within the working range of the atomic absorption spectrometer.]

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Sodium emission line of 589 nm

Lamp: Sodium hollow-cathode

Flame: Oxidizing

Blank: Water

Analysis: Transfer 5.0 mL of the *Sample stock solution* to each of three 25-mL volumetric flasks. To these flasks,

respectively, add 0.0, 5.0, and 10.0 mL of the *Standard stock solution*, dilute with water to volume, and mix. These flasks contain 0.0, 0.20, and 0.40 μg of added sodium/mL, respectively. [NOTE—Concentrations of sodium in these solutions may be modified to fit the linear or working range of the atomic absorption spectrophotometer.]

Determine the absorbances of these solutions. Plot the absorbances of the three solutions versus concentration, in $\mu\text{g}/\text{mL}$ of added sodium, draw the straight line best fitting the plotted points, and extrapolate the line until it intercepts the concentration axis. From the graph determine the concentration, C , in $\mu\text{g}/\text{mL}$ of sodium, of the solution containing 0.0 mL of the *Standard stock solution*.

Calculate the percentage of sodium in the portion of Potassium Nitrate taken by multiplying C by 0.25.

Acceptance criteria: NMT 0.1%

• LIMIT OF NITRITE

Solution A: 1 mg/mL of sulfanilic acid

Solution B: 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride. [NOTE—When stored in a low-actinic glass bottle, this solution may be used for 1 week.]

Standard stock solution: 15 $\mu\text{g}/\text{mL}$ of sodium nitrite (10 $\mu\text{g}/\text{mL}$ of nitrite)

Standard solutions: Transfer 1.0 and 2.0 mL of *Standard stock solution* to separate 50-mL beakers, and add 19 and 18 mL of water to the respective beakers.

Sample solution: Transfer 4.0 g of Potassium Nitrate to a 50-mL beaker, add 20 mL of water, and swirl to dissolve.

Analysis: To the beakers containing the *Standard solutions* and the *Sample solution* add 5.0 mL of *Solution A* and 5.0 mL of diluted hydrochloric acid, and allow to stand for 3 min. Add 5.0 mL of *Solution B* to each beaker, mix, and allow to stand for 15 min. Determine the absorbances of the solutions at 550 nm.

Acceptance criteria: The absorbance of the solution from the *Sample solution* does not exceed that of the solution from the *Standard solution* containing 20 μg of nitrite (5 $\mu\text{g}/\text{g}$).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

Potassium Nitrate Solution

» Potassium Nitrate Solution contains not less than 98.0 percent and not more than 102.0 percent of the labeled amount of KNO_3 .

Packaging and storage—Preserve in tight containers.

Identification—It responds to the tests for *Potassium* (191) and for *Nitrate* (191).

Chloride (221)—An accurately measured portion of Solution, equivalent to 500 mg of potassium nitrate, shows no more chloride than corresponds to 0.21 mL of 0.020 N hydrochloric acid (0.03%, based on the potassium nitrate content of the Solution).

Sulfate (221)—Dilute an accurately measured portion of Solution, equivalent to 100 mg of potassium nitrate, with water to obtain 10 mL of solution, add 15 mL of 6 N hydrochloric acid, and evaporate to dryness on a steam bath. To the residue so obtained add 7 mL of 6 N hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue so obtained in about 35 mL of water and, if necessary, neutralize with hydrochloric acid using litmus paper indica-

tor. Filter, if necessary, to obtain a clear test solution. This test solution shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.1%, based on the potassium nitrate content of the Solution).

Arsenic, Method I (211): 3 ppm, based on the potassium nitrate content of the Solution, an accurately measured portion of Solution, equivalent to 1.0 g of potassium nitrate, being tested.

Lead—Dilute an accurately measured portion of Solution, equivalent to 500 mg of potassium nitrate, with water to obtain 20 mL of test solution. This test solution contains not more than 5 μg of lead (corresponding to not more than 0.001% of Pb, based on the potassium nitrate content of the Solution) when tested as directed under *Lead* (251).

Delete the following:

• **Heavy metals, Method I** (231): 0.002%, based on the potassium nitrate content of the Solution. (Official 1-Jan-2018)

Iron (241): not more than 0.001%, based on the potassium nitrate content of the Solution, an accurately measured portion of Solution, equivalent to 1.0 g of potassium nitrate, being tested.

Limit of sodium—

Stock test solution—Transfer an accurately measured portion of Solution, equivalent to 1.0 g of potassium nitrate, to a 500-mL volumetric flask, dilute with water to volume, and mix. [NOTE—The concentration of potassium nitrate in this solution may be modified by using a different quantity or by further dilution to bring the absorption response within the working range of the atomic absorption spectrophotometer.]

Stock standard solution—Proceed as directed in the *Limit of sodium* test under *Potassium Nitrate*.

Procedure—Proceed as directed in the *Limit of sodium* test under *Potassium Nitrate*. Calculate the percentage of sodium in the portion of Solution taken by multiplying C by 0.25: the limit is 0.1%, based on the potassium nitrate content of the Solution.

Limit of nitrite—

Sulfanilic acid solution, N-(1-Naphthyl)ethylenediamine dihydrochloride solution, and *Standard solutions*—Proceed as directed in the *Limit of nitrite* test under *Potassium Nitrate*.

Test solution—Transfer an accurately measured portion of the Solution, equivalent to 4.0 g of potassium nitrate, to a 50-mL beaker, add sufficient water to obtain 20 mL of solution, and mix.

Procedure—Proceed as directed in the *Limit of nitrite* test under *Potassium Nitrate*. The absorbance of the solution from the *Test solution* does not exceed that of the solution from the *Standard solution* containing 20 μg of nitrite (5 μg per g, based on the potassium nitrate content of the Solution).

Assay—[NOTE—Use water that is carbon dioxide- and ammonia-free.]

Cation-exchange column—Transfer strongly acidic styrene-divinylbenzene cation-exchange resin (16- to 50-mesh) to a 2-cm diameter chromatographic column to a depth of about 20 cm.

Procedure—Transfer an accurately measured portion of Solution, equivalent to about 400 mg of potassium nitrate, to a beaker and add sufficient water to obtain 100 mL of solution. Proceed as directed in the *Assay* under *Potassium Nitrate* beginning with "Pass this solution through" Each mL of 0.1 N sodium hydroxide is equivalent to 10.11 mg of KNO_3 .

Potassium Perchlorate

» Potassium Perchlorate contains not less than 99.0 percent and not more than 100.5 percent of KClO_4 , calculated on the dried basis.

Caution: Great care should be taken in handling Potassium Perchlorate in solution or in the dry state, as explosions may occur if it is brought into contact with organic or other readily oxidizable substances.

Packaging and storage—Preserve in well-closed containers.

Identification—

A: Ignite a small portion of a solution (1 in 10) on a platinum wire in a nonluminous flame: a transient violet color is imparted to the flame.

B: Add a few drops of methylene blue solution (1 in 1000) to the solution (1 in 10): a violet-colored precipitate is formed.

pH (791): between 5.0 and 6.5, in a 0.1 M solution.

Loss on drying (731)—Dry it over silica gel for 12 hours: it loses not more than 0.5% of its weight.

Insoluble substances—Dissolve 20 g in 150 mL of warm water, pass through a tared medium-porosity filtering crucible, and wash with three 50-mL portions of warm water. Dry the residue at 105° for 3 hours: the weight of the residue does not exceed 1 mg (0.005%).

Chloride (221)—A 5.0-g portion shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (0.003%).

Delete the following:

• **Heavy metals, Method I** (231): 0.001%. • (Official 1-Jan-2018)

Limit of sodium—Ignite a small portion of a solution (1 in 10) on a platinum wire in a nonluminous flame: no pronounced yellow color is imparted to the flame.

Assay—

Mobile phase—Transfer 16.6 g of phthalic acid to a 100-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 1000-mL flask, dilute with water to volume, and mix. Adjust with about 450 mg of lithium hydroxide to a pH of 4.5, filter, and degas.

Standard preparation—Transfer about 50 mg of potassium perchlorate, accurately weighed, to a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Using about 50 mg of Potassium Perchlorate, accurately weighed, proceed as directed for the *Standard preparation*.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a conductivity detector and a 4.6-mm \times 7.5-cm column that contains 6- μ m packing L23. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of KClO_4 in the portion of Potassium Perchlorate taken by the formula:

$$500C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of potassium perchlorate in the *Standard preparation*; and r_u and r_s are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Potassium Perchlorate Capsules

» Potassium Perchlorate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of potassium perchlorate (KClO_4).

Packaging and storage—Preserve in tight, light-resistant containers.

Identification—

A: Dissolve the contents of 5 Capsules in 20 mL of water, and filter: the filtrate responds to the tests for *Potassium* (191).

B: Add a few drops of methylene blue solution (1 in 1000) to the filtrate obtained in *Identification test A*: a violet-colored precipitate is obtained.

Disintegration (701): 30 minutes, 1 N hydrochloric acid maintained at $37 \pm 2^\circ$ being used as the immersion fluid.

Uniformity of dosage units (905): meet the requirements for *Content Uniformity*.

Assay—

Mobile phase, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay* under *Potassium Perchlorate*.

Assay preparation—Mix the contents of not less than 10 Capsules, and transfer an accurately weighed portion of the mixed powder, equivalent to about 200 mg of potassium perchlorate, to a 200-mL volumetric flask, dilute with water to volume, and mix. Pass through a filter having 0.22- μ m pore size, transfer 10.0 mL of the clear filtrate to a 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Proceed as directed in the *Assay* under *Potassium Perchlorate*. Calculate the quantity, in mg, of potassium perchlorate (KClO_4) in the portion of Capsules taken by the formula:

$$2000C(r_u / r_s)$$

in which the terms are as defined therein.

Potassium Permanganate

KMnO_4 158.03
Permanganic acid (HMnO_4), potassium salt;
Potassium permanganate (KMnO_4) [7722-64-7].

DEFINITION

Potassium Permanganate contains NLT 99.0% and NMT 100.5% of potassium permanganate (KMnO_4), calculated on the dried basis.

[CAUTION]—Observe great care in handling Potassium Permanganate, because dangerous explosions may occur if it is brought into contact with organic or other readily oxidizable substances, either in solution or in the dry state.]

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Permanganate (191):**
A solution of it is deep violet-red when concentrated and pink when highly diluted.

ASSAY• **PROCEDURE**

Sample: 1000 mg of Potassium Permanganate
Titrimetric system

Mode: Residual titration

Titrant: 0.03 N potassium permanganate VS

Endpoint detection: Visual

Analysis: For each mg of Potassium Permanganate taken, add 2.13 mg of sodium oxalate, previously dried at 110° to constant weight, to a 500-mL conical flask. Add 150 mL of water and 20 mL of 7 N sulfuric acid, and heat to 80°. Titrate the excess oxalic acid with *Titrant*. Calculate the percentage of potassium permanganate (KMnO₄) in the portion of Potassium Permanganate taken:

$$\text{Result} = [(F_1 \times W_S) - (V_S \times N \times F_2)] \times (100/W)$$

F_1 = equivalency factor, 0.4718 mg of potassium permanganate per mg of sodium oxalate

W_S = weight of sodium oxalate taken (mg)

V_S = *Titrant* volume consumed by the *Sample* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F_2 = equivalency factor, 31.61 mg of potassium permanganate per mEq

W = *Sample* weight (mg)

Acceptance criteria: 99.0%–100.5% on the dried basis

IMPURITIES• **INSOLUBLE SUBSTANCES**

Sample: 2.0 g

Analysis: Dissolve the *Sample* in 150 mL of water that previously has been warmed to steam-bath temperature, and filter immediately through a tared, medium-porosity filtering crucible. Wash the filter with three 50-mL portions of the warm water, and dry the filtering crucible and the residue at 105° for 3 h.

Acceptance criteria: 0.2%; NMT 4 mg of residue is obtained.

SPECIFIC TESTS• **LOSS ON DRYING (731)**

Analysis: Dry over silica gel for 18 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

Dibasic Potassium Phosphate

K₂HPO₄

174.18

Phosphoric acid, dipotassium salt;
Dipotassium hydrogen phosphate [7758-11-4].

DEFINITION

Dibasic Potassium Phosphate contains NLT 98.0% and NMT 100.5% of K₂HPO₄, calculated on the dried basis.

IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Potassium (191)**

Sample solution: 1 in 20

Acceptance criteria: Meets the requirements

- **B. IDENTIFICATION TESTS—GENERAL, Phosphate (191)**

Sample solution: 1 in 20

Acceptance criteria: Meets the requirements

ASSAY• **PROCEDURE**

Sample: 5.2 g

Sample solution: Transfer the *Sample* to a 250-mL beaker. Add 50 mL of water and 40.0 mL of 1 N hydrochloric acid, and stir until dissolved.

Blank: Transfer 40.0 mL of 1 N hydrochloric acid to a 250-mL beaker. Add 50 mL of water.

Analysis: Titrate the *Blank* with 1 N sodium hydroxide VS, and record the volume of 1 N sodium hydroxide VS consumed. Titrate the excess acid in the *Sample solution* with 1 N sodium hydroxide VS to the inflection point at pH 4, and record the buret reading. Subtract this buret reading from that of the blank, and designate the volume of 1 N sodium hydroxide VS resulting from this subtraction as A. Continue the titration with 1 N sodium hydroxide VS to the inflection point at pH 8.8, record the buret reading, and calculate the volume (B) of 1 N sodium hydroxide required in the titration between the two inflection points (pH 4–8.8). Where A is equal to or less than B, each mL of the volume A of 1 N sodium hydroxide is equivalent to 174.2 mg of K₂HPO₄. Where A is greater than B, each mL of the volume 2B – A of 1 N sodium hydroxide is equivalent to 174.2 mg of K₂HPO₄.

Acceptance criteria: 98.0%–100.5% on the dried basis

IMPURITIES• **INSOLUBLE SUBSTANCES**

Sample solution: Dissolve 10 g in 100 mL of hot water.

Analysis: Filter the *Sample solution* through a tared filtering crucible, wash the insoluble residue with hot water, and dry at 105° for 2 h.

Acceptance criteria: The weight of the residue is NMT 20 mg (NMT 0.2%).

• **CARBONATE**

Sample: 1 g

Analysis: Add 3 mL of water and 2 mL of 3 N hydrochloric acid to the *Sample*.

Acceptance criteria: NMT a few bubbles are evolved.

• **CHLORIDE AND SULFATE, Chloride (221)**

Sample: 1.0 g

Acceptance criteria: Shows no more chloride than corresponds to 0.40 mL of 0.020 N hydrochloric acid (NMT 0.03%)

• **CHLORIDE AND SULFATE, Sulfate (221)**

Sample: 0.20 g

Acceptance criteria: Shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (NMT 0.1%)

• **ARSENIC, Method I (211):** NMT 3 ppm• **IRON**

Sample solution: Dissolve 0.33 g in 10 mL of water.

Analysis: To the *Sample solution* add 6 mL of hydroxylamine hydrochloride solution (1 in 10) and 4 mL of orthophenanthroline solution prepared by dissolving 1 g of orthophenanthroline in 1000 mL of water containing 1 mL of 3 N hydrochloric acid, and dilute with water to 25 mL.

Acceptance criteria: Any red color produced within 1 h is not darker than that of a control prepared from 1 mL of *Standard Iron Solution* (see *Iron (241)*): NMT 30 ppm.

- **SODIUM:** A solution (1 in 10) tested on a platinum wire imparts no pronounced yellow color to a nonluminous flame.

Delete the following:• **HEAVY METALS, Method I (231)**

Sample stock solution: Dissolve a portion equivalent to 4.2 g of K_2HPO_4 in enough water to make 50 mL.

Analysis: Transfer 12 mL of the *Sample stock solution* to a 50-mL color-comparison tube (*Test Preparation*).

Transfer 11 mL of the *Sample stock solution* to a second color-comparison tube containing 1.0 mL of *Standard Lead Solution* (*Monitor Preparation*). Transfer 1.0 mL of *Standard Lead Solution* and 11 mL of water to a third color-comparison tube (*Standard Preparation*). Proceed as directed for *Procedure*, omitting the dilution to 50 mL.

Acceptance criteria: NMT 10 ppm • (Official 1-Jan-2018)

• **LIMIT OF FLUORIDE**

[NOTE—Prepare and store all solutions in plastic containers.]

Buffer: 294 g/L of sodium citrate dihydrate in water

Standard stock solution: 1.1052 mg/mL of USP Sodium Fluoride RS in water

Standard solution: Transfer 20.0 mL of *Standard stock solution* to a 100-mL volumetric flask containing 50.0 mL of *Buffer*, and dilute with water to volume. Each mL of this solution contains 100 µg of fluoride ion.

Sample: 2.0 g

Electrode system: Use a fluoride-specific ion-indicating electrode and a silver-silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ± 0.2 mV (see pH (791)).

Standard response line: Transfer 50.0 mL of *Buffer* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.

Analysis: Transfer the *Sample* to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer* and sufficient water to make 100 mL (*Sample solution*). Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard response line* determine the concentration, C , in µg/mL, of fluoride ion in the *Sample solution*. Calculate the percentage of fluoride ion in the portion of Dibasic Potassium Phosphate taken:

$$\text{Result} = (C/C_U) \times 100$$

C = concentration of fluoride ion in the *Sample solution* (µg/mL), as defined above

C_U = concentration of the *Sample solution* (µg/mL)

Acceptance criteria: NMT 0.001%

• **LIMIT OF MONOBASIC OR TRIBASIC SALT**

Sample solution: Dissolve 3 g in 30 mL of water, and cool to 20°.

Analysis: Add 3 drops of thymol blue TS to the *Sample solution*.

Acceptance criteria: A blue color is produced, which is changed to yellow (with a greenish tinge) by the addition of NMT 0.4 mL of 1 N hydrochloric acid.

SPECIFIC TESTS

• **pH (791):** 8.5–9.6, in a solution (1 in 20)

• **Loss on Drying (731):** Dry a sample at 105° to constant weight: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**
USP Sodium Fluoride RS

Potassium Phosphates Injection

» Potassium Phosphates Injection is a sterile solution of Monobasic Potassium Phosphate and Dibasic Potassium Phosphate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of monobasic potassium phosphate (KH_2PO_4) and dibasic potassium phosphate (K_2HPO_4). It contains no bacteriostat or other preservative.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass.

Labeling—The label states the potassium content in terms of milliequivalents in a given volume, and states also the elemental phosphorus content in terms of millimoles in a given volume. Label the Injection to indicate that it is to be diluted to appropriate strength with water or other suitable fluid prior to administration, and that once opened any unused portion is to be discarded. The label states also the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

USP Reference standards (11)—

USP Endotoxin RS

Identification—It responds to the tests for *Potassium* (191) and for *Phosphate* (191).

Bacterial Endotoxins Test (85)—It contains not more than 1.10 USP Endotoxin Units per mg of potassium phosphates.

Particulate Matter in Injections (788): meets the requirements for small-volume Injections.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* (1).

Assay for monobasic potassium phosphate—Transfer an accurately measured volume of Injection, equivalent to about 300 mg of monobasic potassium phosphate, to a 100-mL beaker, and dilute with water to about 50 mL. Place the electrodes of a suitable pH meter in the solution, and titrate with 0.1 N sodium hydroxide VS to the inflection point to a pH of about 9.1. Each mL of 0.1 N sodium hydroxide is equivalent to 13.61 mg of KH_2PO_4 .

Assay for dibasic potassium phosphate—Transfer an accurately measured volume of Injection, equivalent to about 300 mg of dibasic potassium phosphate, to a 100-mL beaker, and dilute with water to about 50 mL. Place the electrodes of a suitable pH meter in the solution, and titrate with 0.1 N hydrochloric acid VS to the inflection point to a pH of about 4.2. Each mL of 0.1 N hydrochloric acid is equivalent to 17.42 mg of K_2HPO_4 .